VOLUME LV NUMBER 2

MEDICAL LIBRARY AUG

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

August, 1934

MEDICAL LIBRARY

Pneumococcal Lipoid Nephrosis and the Relation Between Nephrosis and Nephritis. II. Experimental Studies. S. S. BLACKMAN, JR. 85
Insomnia in Clinic Psychiatric Practice. WENDELL MUNCIE, M.D. 131
Urobilin Excretion of Infancy and Childhood; Relation to Blood Destruction and Formation. HUGH W. JOSEPHS. . 154
Book Reviews 169

Supplement—BULLETIN OF THE INSTITUTE OF THE HISTORY OF MEDICINE

THE JOHNS HOPKINS UNIVERSITY
VOLUME II, NUMBER 6

William H. Welch. HENRY E. SIGERIST 345
Tenth-Century Medicine as seen in the Historia of Richer of Rheims. L. C. MACKINNEY 347
Texts and Documents
Medical Dissertation on Nostalgia by JOHANNES HOFER, 1688. CAROLYN KISER ANSPACH 376
Notes and Comments
On Some Further Editions of Faust's "Catechism of Health." HENRY E. SIGERIST 392
Activities of the Institute of the History of Medicine 402

PUBLISHED MONTHLY
THE JOHNS HOPKINS PRESS, Baltimore, Maryland
Made in United States of America

BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

MANAGING EDITOR, E. Cowles Andrus

EDITORIAL BOARD

Charles R. Austrian
Thomas R. Boggs
Edward P. Carter
Arnold R. Rich
Paul G. Shipley
Frank R. Smith

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore, Md.

Books for review should be sent to The Editor at the above address.

Twenty-five reprints, without covers, of articles will be furnished to contributors free of cost. An order slip for additional reprints, with a table showing cost, is sent with proof.

Correspondence concerning business matters should be sent to The Johns Hopkins Press, Baltimore, Maryland, U. S. A.

This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro rata value of such numbers will be credited to the next volume, and the renewal invoice therefore adjusted accordingly.

Subscriptions should be renewed promptly. To avoid a break in your series, subscriptions should be renewed promptly. The publisher cannot guarantee to supply back issues on belated renewals.

Subscriptions, new or renewal, may be sent to the Publishers or ordered through your agent.

Agents for the British Empire, except Canada: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, W.C. 2, London.

THE JOHNS HOPKINS PRESS
BALTIMORE, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918

PNEUMOCOCCAL LIPOID NEPHROSIS AND THE RELATION BETWEEN NEPHROSIS AND NEPHRITIS

II. Experimental Studies

S. S. BLACKMAN, JR.

From the Department of Pathology, The Johns Hopkins University, Baltimore, Md.

Submitted for publication January 25, 1934

CONTENTS

1. Pneumococcal toxin 86
   (a) Production and stability 87
   (b) On the chemical nature of toxin 88
   (c) Variation in the susceptibility of animal species 89
   (d) The minimal lethal dose for rabbits 90
   (e) The effect of lethal doses on the tissues, blood and temperature of rabbits without immunity 90
   (f) The antigenic properties of toxin and the duration of immunity 101
2. Experimental pneumococcal nephritis and nephrosis 105
   (a) Acute tubular nephritis 107
   (b) Acute and progressive glomerulonephritis 107
   (c) Experimental nephrosis 112
      (c 1) Methods and material 112
      (c 2) Duration 115
      (c 3) The urine 116
      (c 4) The non-protein nitrogen and cholesterol of the blood 116
      (c 5) Plasma proteins and oedema 117
      (c 6) The kidneys and other tissues 118
      (c 7) The treatment of experimental nephrosis with antitoxin 120
3. Comparison between the effects of pneumococcal toxin on the tissues of man and animals 120
4. Discussion and Conclusions 121
   (a) The relation between nephrosis and nephritis 121
   (b) Problems in the pathogenesis of nephrosis and nephritis caused by bacterial toxins 123
   (c) Conclusions 125

In the past lipoid nephrosis has often been considered to be fundamentally different from glomerulonephritis, both in clinical manifestations and pathological characteristics, and also in aetiology. Although there are definite and important distinctions between the two diseases,

data have recently been described which indicate that these distinctions depend on the occurrence of pathological changes in the function and histological structure of the kidney which are identical in kind, but different chiefly in degree (1). It has been shown, for example, that the most characteristic differences between lipoid nephrosis and glomerulonephritis, the absence of hypertension and progressive nitrogen retention in the blood in nephrosis, are intimately related to the absence of diffuse obliteration of glomeruli. It was further pointed out that the conversion of glomeruli to scar tissue in glomerulonephritis results from the organization of clots of exudate in the glomerular capsules. The absence of fibrinous clots in the glomerular capsules in nephrosis constitutes the one clearly demonstrable and fundamental histological difference between nephrosis and glomerulonephritis. Other functional changes (oliguria, albuminuria, haematuria) and histological lesions (cloudy swelling, fat and lipoid deposits in glomerular and tubular epithelium, necrosis of epithelial cells, and regeneration of glomerular and tubular epithelium) occur in both diseases in different degrees. There is evidence, too, that lipoid nephrosis, like glomerulonephritis, is commonly caused by chronic bacterial infection, and it appears that chronic pneumococcal infection is particularly effective in causing nephrosis in children, in whom the disease most often occurs.

The experimental data described in the following pages lend added support to these views regarding the nature and aetiology of nephrosis. Small quantities of pneumococcal toxin, injected intravenously into rabbits, cause an experimental disease like nephrosis, without the formation of either clots of exudate in glomerular capsules or destructive lesions of the glomerular capillaries. Acute and progressive glomerulonephritis, with typical clinical and histological characteristics, may be reproduced in these animals by means of larger doses of the same toxin.

Before experimental pneumococcal nephritis of various forms is described in detail, however, it is necessary to give some account of the properties of pneumococcal toxin, and to state the effects produced by it in different laboratory animals.

1. PNEUMOCOCCAL TOXIN (AUTOLYSATE)

There is no full account of the properties of pneumococcal toxin (autolysate), and of its action on the tissues of animals. It is well

known that *in vitro* the toxin causes haemolysis of red blood cells (2), and digestion of proteins, carbohydrates, and fats (3). It is familiar, too, that in mice the toxin causes purpuric haemorrhages (4), while local injections into the skin and lungs of guinea-pigs produce necrosis of tissue and inflammation (5). Many pneumococcal toxins are therefore assumed to exist. The haematoxin, the purpura-producing principle, the guinea-pig skin and lung necrotizing toxins, and names of various "enzymes" are all more or less familiar.

The autolysate is certainly a complex mixture. It seems unlikely, however, that each effect in every tissue in different species of animals is produced by a separate toxin. The corresponding tissues in different animals are not equally susceptible to damage by pneumococcal toxin, and experiments are still in progress to determine whether or not there exist many different toxins. For the sake of clarity in this paper, therefore, the properties and action characteristic of pneumococcal toxin (autolysate), as observed in experiments carried out during the past several years and still in progress, are briefly summarized.

(*a*) *Production and Stability*

The following descriptions of the properties and action of pneumococcal toxin apply specifically to that made from Type I Pneumococci, Neufeld Strain. Whether or not there are any differences in the toxins of Pneumococci of various types has not been carefully investigated. In a small number of experiments with toxin obtained from Type II and Type III Pneumococci, however, the lesions produced in the tissues of rabbits have been similar to those caused by the toxin of Type I Pneumococci.

The toxin used in the experiments described here was made after the method reported by Parker (5). The procedure has differed in some details. The organisms are grown for 24 hours in 200 cc. bottles of double-strength veal-peptone broth. The concentrated bacteria are collected by centrifugalizing at high speed for one hour, and may then be suspended in test-tubes of fresh broth, salt solution, or distilled water. Approximately 4 cc. of pneumococci are suspended in a volume of 12 cubic-centimetres. The test-tubes are sealed with vaseline. After ten to fourteen days at room temperature in the dark the contents of the tubes become more or less clear, depending on how

many bacteria have autolyzed. After filtering through Berkefeld candles, the clear, sterile, filtrate contains the toxin. The degree of autolysis of the bacteria varies from time to time for reasons which have not been thoroughly investigated. When made in this way, sealed with vaseline, and stored in the refrigerator for over a year, the toxin has been found to retain its original strength.

In experiments with guinea-pigs, Parker (5) found the toxin very labile, and extremely sensitive to air, light, and heat. Concerning the effect of oxygen it may be said that after passing pure oxygen gas through the toxin for one-half hour, its potency in causing albuminuria and lesions in the tissues of rabbits is undiminished.

Heat gradually destroys the toxin. Minimal lethal intravenous doses for rabbits are still toxic after being heated to 37° and 50°C. for half-hour periods. Similar doses heated to 80°C. are practically harmless for rabbits. But even after boiling the toxin, mice are somewhat susceptible to it.

The strength of the toxin is diminished by repeated filtration, and this is of some importance, since it suggests that the poisons formed in the tissue of man during infection, by autolysis of the bacteria, may be more potent than the filtered autolysate. After a second filtration of the toxin through Berkefeld candles, quantities four times as large as the minimal lethal dose are practically harmless for rabbits. Minimal lethal doses of the original filtrate produced albuminuria at the rate of 25 grams per liter. After a second filtration single intravenous injections equal to four minimal lethal doses produced at the most one gram of albumin per liter of urine.

(*b*) *On the Chemical Nature of Toxin*

The actual chemical nature of pneumococcal toxin described above is still uncertain. The experiments carried out so far suggest that it is a protein, for when the autolysate is filtered through an ultrafilter, a Berkefeld candle saturated with a collodion solution, and dried, the residue retains the active toxin and gives positive tests for protein. The filtrate contains salts, carbohydrate, and nitrogen. It gives none of the positive tests for protein, and is harmless for rabbits in causing albuminuria, which is apparently the most delicate test in rabbits of the strength of the toxin. Since also the toxin is actively antigenic,

as is well known, there are reasons to believe that it consists of large molecules, probably protein in nature.

(c) *The Variation in Species Susceptibility*

Pneumococcal toxin damages many tissues of mice, guinea-pigs, rabbits and dogs. In different species, however, similar tissues are not equally affected. In all of these animals the tissues especially damaged are the blood capillaries, the kidneys, and voluntary and cardiac muscles. As a result, albuminuria, the lesions of some form of nephritis, and focal areas of necrosis in some muscles develop.

In mice the most conspicuous gross lesions are the numerous subcutaneous purpuric haemorrhages which are perfectly familiar. Haemorrhages occur especially in the heart, in some voluntary muscles, and from the glomeruli of guinea-pigs.

The red blood cells of dogs are rapidly destroyed even by small doses of toxin. Within five days two intravenous injections of 2 cc. each may reduce the red blood cells of a dog weighing six kilograms from 6,000,000 to 800,000 per cubic millimeter. Larger doses of toxin given to dogs produce widespread haemorrhages in the subcutaneous tissues, stomach and intestine, liver, spleen, testis, kidneys, brain, and in the cardiac and voluntary muscle, with little or no visible necrosis of muscle fibres. There may be extensive central and midzonal necrosis of cells in the liver, and this may occur within a few hours, before the development of anaemia. Dogs regularly become jaundiced, which is not surprising in the presence of both anaemia and necrosis of liver cells. Extensive haemorrhage into the pulp of the dog's spleen occurs, and similar lesions are occasionally found in the spleens of rabbits given toxin.

In dogs, moderate albuminuria (1 to 6 gms. per liter), decreasing plasma protein (3.66 gms.%), inverted albumin-globulin ratio, lipaemia (cholesterol 240 mgms.%), and high blood non-protein nitrogen may all be produced with ease and regularity.

The lesions which are caused by the toxin in the tissues of rabbits are described in detail below. It should be said here, however, that the only haemorrhages which have been seen in rabbits are those from the glomeruli of the kidneys, occasional small haemorrhages in the spleen and lungs, and, rarely, haemorrhages in muscle in association

with foci of necrosis. In contrast to the effect of the toxin on the kidneys of dogs, massive albuminuria is produced in rabbits, 30 grams per liter within 48 hours.

(*d*) *The Minimal Lethal Dose for Rabbits*

For rabbits the minimal lethal dose of toxin used in these experiments is the smallest quantity, injected intravenously, which killed the majority of the test animals, four out of six, within five days. The minimal lethal dose of the strongest toxin used was 0.1 cc. of toxin per 100 grams weight of rabbit. The minimal lethal dose of the weakest toxin was 0.2 cc. of toxin per 100 grams weight of rabbit. Since the strength varies with the degree of autolysis of the bacteria, the toxin must be standardized with each production.

When injected subcutaneously into rabbits, local damage of tissue is produced, accompanied by inflammation which is characterized by very massive oedema and comparatively slight redness of the tissues. Some of the toxin injected is absorbed from the site, and albuminuria and lesions in the kidneys and other organs affected by the toxin may be produced.

(*e*) *The Effect of Lethal Doses in Rabbits Without Immunity*

The Kidneys. When single lethal doses of toxin are given, rabbits usually die within five days. A few hours after the toxin is injected urine may be excreted containing variable amounts of albumin. The sooner the urine is excreted the smaller is the albumin content, which is at most a few grams per liter, or less, at this time. Within twenty-four to forty-eight hours after this specimen has been excreted there is usually either very marked oliguria or complete anuria. If urine is excreted it contains from 20 to 30 grams of albumin per liter, sometimes more, and there are many large coarse granular casts, and some leukocytes in the centrifugalized sediment. Gross haematuria has not been observed to follow single lethal doses of toxin. Microscopically, in the sediment, very moderate numbers of red blood cells may be found.

After this period, if the animal survives longer, the volume of urine excreted in 24 hours increases, and the albumin, casts, and red blood cells, if formerly present, decrease gradually and the urine becomes

TABLE I

"Tubular" nephritis following a single injection of toxin

EXPERIMENT NUMBER	WEIGHT OF RABBIT	DURATION OF EXPERIMENT	DOSAGE OF TOXIN		BLOOD CHEMISTRY AT END OF EXPERIMENT						FLUIDS		ALBUMINURIA		FLUID IN TISSUES AND BODY CAVITIES		
			Number of doses	Total quantity given	N.P.N.	Cholesterol	Plasma protein	Albumin nitrogen	Globulin nitrogen	A/G ratio	Total volume of fluid injected	Total volume of urine excreted	Grams per liter (average, Esbach)	Total albumin excreted	Oedema	Hydrothorax	Ascites
	grams			*cc.*	*mgm. %*	*mgm. %*	*grams %*	*grams %*	*grams %*		*cc.*	*cc.*		*grams*			
31-1	1,520	6½ hrs.	1	3.0	40	57	4.09	0.397	0.258	61/39	0	*	32		0	0	0
58-4	1,520	12 hrs.	1	1.0	56	107	4.73	0.455	0.302	60/40	0	66	3.25	0.132	0	0	0
1-9	1,020	18 hrs.	1	1.0	85	107	2.59	0.245	0.170	59/41	0	*	2		0	0	0
58-1	1,500	21 hrs.	1	1.5	72	130	3.50	0.253	0.307	45/55	0	110	9	0.990	0	0	0
1-8	1,040	24 hrs.	1	1.0	116	166	3.02	0.349	0.135	58/42	0	0			0	0	0
1-6	960	29 hrs.	1	0.96	136	187	3.35	0.171	0.365	32/68	0	*	2.0		+	0	0
2-2	900	30 hrs.	1	0.90	160	230	3.25	0.042	0.478	8/92	0	*	30.0		+	0	0
1-2	850	36 hrs.	1	0.85	176	187	2.77	0.146	0.298	33/67	0	8	3.0	0.024	+	0	0
56-18	2,480	4 days	1	3.5	310	167	3.48	0.282	0.275	51/49	100	400	7.0	2.800	+	4 cc.	15 cc.; albumin, 0.8%

* Not measured.

entirely normal within a few days. Oedema of the subcutaneous tissues may not develop, but usually appears on the third day. Occasionally it has been seen within 48 hours after the toxin is injected. Some animals develop diarrhoea which may disappear after one or two days, or may persist until death. In these the urine may be obtained by slowly compressing the bladder.

The changes which occur in the blood, the elevation of cholesterol and non-protein nitrogen, and the loss of plasma protein, are shown in table I. These animals were bled from the heart and killed at various intervals following the injection of a single dose of toxin.

The lesions in the kidneys vary with the duration of the nephritis, and depend also on the quantity of toxin given. Single minimal lethal doses cause necrosis of glomerular epithelium and of the epithelium in patches of tubules. Definite necrosis of some extent is usually plainly demonstrable after thirty hours. The cells in many of the tubules show no more than cloudy swelling, and in some tubules the cells may show "colloid" droplets of cytoplasm. After longer intervals the necrotic cells may be found as calcified debris within the tubules, while many more tubules are filled with hyaline casts. The regenerated epithelial lining of the tubules which have undergone necrosis consists of flat cells which stain deeply.

Larger single doses than the minimal lethal dose cause more widespread necrosis of the epithelium which may be demonstrable after six hours. The glomerular epithelium is affected like that of the tubules, although the changes are more difficult to see plainly and need to be looked for carefully. Regenerating glomerular epithelium can be recognized in Bowman's capsule, and over the glomerular capillaries.

Most of the glomerular capillaries themselves are not histologically altered. In the animals given single minimal lethal doses the capillaries look normal usually. In an occasional animal there may be a few widely scattered fibrin thrombi in some of the capillaries, and occasional haemorrhages in the tubules may be found. These changes, capillary thrombi and haemorrhages, are found somewhat more frequently when single doses of toxin are given exceeding the minimal lethal dose.

The Blood Capillaries. It is obvious from the degree of albuminuria which follows the injection of a single minimal lethal dose of toxin

that the glomerular capillaries are severely damaged. The following experiment shows that the toxin is a general capillary poison as well. It will be seen, first, that following injection of toxin, loss of plasma protein may occur which cannot be entirely accounted for by the quantity of albumin excreted in the urine. Secondly, since at this time there is oedema, and ascites containing albumin almost equal in percentage to that of the urine, it is self-evident that plasma protein escapes through both the glomerular and general tissue capillaries. From this evidence it may be concluded that the toxin is a general capillary poison and that the oedema and ascites are dependent on capillary damage to the extent that the latter is the direct cause of the escape of albumin from the blood plasma into the tissues and body cavities.

Expt. 55. R-1. Weight, 1240 gms. Over a period of 60 hours this rabbit was given 2.5 cc. of pneumoccal toxin in three intravenous injections. During this time 16 cc. of urine were excreted containing an average of 14.08 gms. of albumin per liter, or 1.408%, a total of 0.225 gm. of albumin. At the end of 60 hours the animal was given ether, bled from the heart, and killed. At autopsy the urinary bladder was empty. The subcutaneous tissues about the sacrum and thighs were markedly swollen with oedema. Each pleural cavity contained 2–3 cc. of clear fluid. From the abdominal cavity 5 cc. of fluid were removed which contained 11 grams of albumin per liter, or 1.1%. The total protein of the blood plasma was low, being 3.16 gms. per cent. Both the albumin and globulin fractions of the total protein were decreased (albumin nitrogen, 0.277 and globulin nitrogen 0.228), so that the ratio was practically normal.

Plasma Protein and Oedema. It has been shown above that the pneumococcal toxin causes a decrease in the protein content of the blood plasma by virtue of the escape of protein through damaged glomerular and general tissue capillaries. The experiments summarized below further illustrate the importance of general capillary damage as a part of the mechanism responsible for the loss of protein from the blood plasma, and also indicate that the escape of plasma protein into the tissue *per se* plays an important rôle in the pathogenesis of the collection of *fluid* in the tissues. Since the total plasma protein may be low and the albumin-globulin ratio inverted before oedema is grossly demonstrable, and since oedema first appears in dependent parts of the body, these data suggest that the earliest *demonstrable* presence of oedema is the result of the progressive escape of small

amounts of albumin in solution through a wide capillary bed, eventually to appear by gravity in dependent parts.

By determining the total quantity of plasma protein at different intervals after toxin is injected into rabbits, its progressive fall may be followed. The experiments carried out in this way show that the plasma protein may reach the so-called critical level for the development of oedema, and the albumin-globulin ratio may be inverted, before hydrothorax, ascites, or oedema is demonstrable. Since the fluid which accumulates in the tissues and body cavities, when its presence can be detected at a later period, contains albumin nearly equal in percentage to that in the urine, the question to be raised is, therefore, not so much the relation between low plasma protein and the development of oedema, but rather the relation between the presence of albumin in the tissue spaces and the accumulation of fluid in the tissues and body cavities. The passage of albumin through damaged capillaries into the tissue spaces *may* cause water to collect in two ways. It is obvious, first, that albumin can escape from the blood into the tissues only in solution, *i.e.*, albumin and fluid must pass through the capillaries together. The first step in the formation of oedema, therefore, may be capillary damage which allows plasma proteins in solution to escape into the tissue spaces. Secondly, since it is generally admitted that molecules of albumin above all others attract water by exerting a high osmotic pressure, at the first appearance of albumin in the tissues, before oedema is grossly demonstrable, it might be considered that the rising osmotic pressure in the tissue spaces must immediately tend to retain water in the tissues. The accumulation of fluid will later continue to progress as the osmotic pressure of the blood plasma falls on account of constant loss of albumin through damaged capillaries into the urine and tissue spaces. As more fluid collects the albumin will be diluted, and this will account for the low osmotic pressure obtained in samples of transudates reported by Schade and Clausen (6), and by Iverson (7). In the early stages of oedema formation, however, when the albumin in the tissue spaces is concentrated, it may exert a more powerful osmotic pressure and therefore retain fluid. In fact, it has been shown by Verney (8) and by Fishberg (9) that plasma proteins do exert more osmotic pressure in concentrated than in dilute solutions.

The following experiments show (1) that ascites may develop rapidly, while the total plasma proteins are still high, and be entirely independent, therefore, of low osmotic pressure in the blood plasma. They illustrate (2) the rôle of the escape of plasma proteins into the tissue spaces in the pathogenesis of oedema, and (3) suggest that plasma proteins collect in the tissues *before the presence of gross fluid is demonstrable*, which may depend largely on the time required for diffuse tissue oedema to collect by gravity in dependent parts of the body.

Expt. 1. Duration, 24 hours. Result: Ascites developed without low total plasma proteins. R. 34–27. White rabbit weighing 1340 grams. Given one dose of toxin intravenously, 0.3 cc. Urine: the total volume excreted in 24 hours was 128 cc. It contained an average of 2.25 gms. of albumin per liter, or a total of 0.256 gram of albumin. Autopsy: No oedema could be recognized. In the abdominal cavity there were 5 cc. of fluid, containing 0.6 gm.% of protein. Blood: Total plasma protein, 4.72 gms.%; albumin nitrogen, 0.558; globulin nitrogen, 0.198; A/G ratio, 74/26.

Expt. 2. Duration, 19 hours. Result: No oedema. R. 56–21. White rabbit, 2100 gms. in weight. Given one intravenous injection of 3.5 cc. of toxin. Urine: None excreted. Autopsy: No demonstrable oedema or ascites. In the bladder there were 2 cc. of urine, containing 10 gms. of albumin per liter, a total of 0.02 gm. Blood: Total plasma protein, 5.42 gms.%; albumin nitrogen, 0.474; globulin nitrogen, 0.393; A/G ratio, 55/45.

Expt. 3. Duration, 26 hours. Result: Plasma protein low. A/G ratio inverted. No demonstrable oedema. R. 12–5. White rabbit, 1650 gms. in weight. Given 3 cc. of toxin in two doses. Urine: 8 hours after the first dose of toxin, 18 cc. of urine were excreted, containing 0.5 gm. of albumin per liter. In the bladder at autopsy there were 5 cc. of urine, containing 20 gms. of albumin per liter. The total volume of urine excreted in 26 hours was 23 cc., containing an average of 10.25 gms. of albumin per liter, or 1.025%. The total amount of albumin excreted was therefore 0.23 gram. Autopsy: There was no demonstrable oedema or ascites. Blood: Total plasma protein, 3.89 gms. %; albumin nitrogen, 0.265; globulin nitrogen, 0.358; A/G ratio, 42/58.

Expt. 4. Duration, 28 hours. Result: Plasma proteins low. A/G ratio inverted. No demonstrable oedema. R. 12–4. White rabbit, weighing 1650 gms. Given one intravenous injection of toxin of 2 cc. Urine: 23 cc. were excreted containing 4 gms. of albumin per liter. At autopsy there were 5 cc. of urine in the bladder containing 30 gms. of albumin per liter. In all, 28 cc. were excreted in 28 hours. The average albumin content was 17 gms. per liter, or 1.7%. The total amount of albumin excreted in the urine was 0.476 gm. Autopsy: There was no demonstrable oedema or ascites. Blood: Total plasma protein, 3.56 gms.%; albumin nitrogen, 0.191; globulin nitrogen, 0.379; A/G ratio 33/67.

Expt. 5. Duration, 72 hours. Result: Plasma protein low. A/G ratio not inverted. Oedema and ascites present. R. 34–25. White rabbit, weighing 1020 gms. Given one intravenous injection of toxin, 0.45 cc. Urine: The total volume excreted was 270 cc., containing an average of 3.83 gms. of albumin per liter and a total of 1.034 grams of albumin. Autopsy: The tissues about the sacrum and the subcutaneous tissues of the thigh were slightly oedematous. The thoracic cavity contained 6 cc. of fluid, and the abdominal cavity 25 cc. of fluid. The albumin content of the ascitic fluid was 0.2 gm.%. Blood: Total plasma protein, 3.56 gms.%; albumin nitrogen, 0.316; globulin nitrogen, 0.257; A/G ratio, 55/45.

From experiments 4 and 5 the importance of the time required for fluid to accumulate in the tissues will be seen. In experiment 4, at the end of 28 hours, no oedema was demonstrable, though the plasma protein of the animal was 3.56 gm. per cent, the same as the plasma protein of the animal in experiment 5 after 72 hours, when both oedema and ascites were present.

Cholesterolaemia. One of the most characteristic results which usually follows the intravenous injection of toxin into rabbits (as well as dogs) is the prompt elevation of the blood cholesterol, as shown in table I. The other lipoids of the blood, neutral fats, fatty acids, etc. have not been investigated in these experiments. When the blood cholesterol is elevated, it is obvious from the gross appearance of the plasma, which usually has an opaque milky appearance.

None of the theories which have been offered satisfactorily explains the pathogenesis of the high blood cholesterol which occurs in nephrosis and nephritis in man. Theoretically, it may be due to (1) increased ingestion, (2) decreased excretion by the normal channels, (3) failure of utilization of lipoids formed at the normal rate, (4) to an increased rate of formation, or to a combination of these.

There is not much evidence to support the idea that the lipaemia of nephritis in man is caused by increased ingestion, and certainly this cannot be the explanation in experimental nephritis and nephrosis in rabbits, since within twelve hours after a single dose of toxin the blood cholesterol may be already elevated.

In man the excretion of cholesterol in the bile in nephrosis has not apparently been investigated. Work which is now in progress indicates that in the dog with experimental bile fistula the cholesterolaemia produced by means of pneumococcal toxin is not dependent upon diminished excretion of cholesterol in the bile. Since there is

said to be little cholesterol excreted in the normal urine of man and rabbits, it is unlikely that diminished excretion in the urine is responsible for the characteristic cholesterolaemia of nephrosis.

As for failure of utilization of lipoids, it may be said that the investigations which have been made tend to show that there is no marked disturbance in fat metabolism. Hiller, Linder, Lundsgard and Van Slyke (10), found that, after feeding fat including cholesterol to patients with nephrosis and nephrotic types of nephritis, the total metabolism and the non-protein respiratory quotient were both normal, and there was no elevation of the blood cholesterol.

There is evidence, however, in favor of the idea that lipaemia in these diseases may be related to some abnormality in the rate and manner of formation of certain lipoids. Avery and Cullen (3) have shown that pneumococcal toxin, acting *in vitro* on a solution of tributyrin, has a marked lipolytic activity, measured by the amount of fatty acid liberated in its action on this ester. It is well established that in nephrosis and nephrotic types of nephritis the fatty acids of the blood, as well as the cholesterol, are elevated (11). These data suggest that, at least in experimental pneumococcal nephrosis and nephritis, digestion of tissue by the toxin, and the consequent accumulation in the blood of the products of this digestion, may be one factor in the mechanism responsible for the lipaemia. Further evidence that abnormal accumulation of cholesterol is concerned in the mechanism responsible for the cholesterolaemia is given below with the description of experimental progressive nephritis, and this mechanism is suggested also by the data in table II. In this series of 10 rabbits nephritis was produced, and the animals were killed at intervals varying from 18 hours to 23 days. In successive animals increasing total dosage of toxin was given. Nevertheless, in the experiments from 4 to 23 days in duration, there was a progressive increase of immunity to the effects of the toxin, manifested clinically and anatomically. Albuminuria and oedema decreased or disappeared. The non-protein nitrogen of the blood, the plasma proteins and cholesterol returned towards or to normal. Histologically, the kidneys show decreasing necrosis of epithelium, or no necrosis in the longer experiments. In these experiments, the doses of toxin used were insufficient to cause widespread formation of clots of exudate within the glomerular capsules, or other destructive lesions

of the glomerular capillaries. There was, therefore, no retention of non-protein nitrogen in the blood in the longer experiments, and the elevated non-protein nitrogen of the blood in the short experiments was associated with relative anuria.

In the longer experiments the decreasing albuminuria and necrosis of renal epithelium seem to be the results of increasing immunity. Since there is no evidence to indicate that cholesterolaemia depends on the failure of excretion, the fall of blood cholesterol towards and to normal in the longer experiments, parallel with decreasing albuminuria and necrosis of tissues, suggests that the rapid development of choles-

TABLE II

Illustrating the decrease in cholesterolaemia parallel with progressive rise of immunity

EXPERIMENT NUMBER	DURATION OF EXPERIMENT	TOTAL QUANTITY OF TOXIN INJECTED	NUMBER OF DOSES	PLASMA CHOLESTEROL ON LAST DAY OF EXPERIMENT	BLOOD N.P.N. ON LAST DAY OF EXPERIMENT	ALBUMINURIA ON LAST DAY OF EXPERIMENT
		cc.		*mgm. %*	*mgm. %*	*grams per liter*
1-9	18 hrs.	1	1	107	85	2
58-1	21 hrs.	1.5	1	130	72	9
2-2	30 hrs.	0.9	1	230	160	30
55-1	60 hrs.	2.5	3	230	260	21
56-18	4 days	3.5	1	167	310	2
34-30	6 days	3.5	11	79	30	1
57-5	8 days	7.0	8	109	43	7
34-39	12 days	13.1	23	60	39	0.25
57-1	23 days	64	42	83	36	0.5
56-2	23 days	64	42	71	35	+

terolaemia in normal rabbits with no immunity represents a direct effect of toxin on the tissues, resulting in abnormal production or liberation of cholesterol, just as necrosis of cells and albuminuria are caused by the toxin acting on the renal epithelium and glomerular capillaries, respectively.

Non-Protein Nitrogen of the Blood. Following the injection of a single lethal dose of toxin, marked oliguria or anuria usually develops, and there is a progressive elevation of the non-protein nitrogen of the blood, probably dependent at this time on glomerular damage and suppression of urine. It may in part, however, be the result of other mechanisms which have not as yet been fully investigated, such as the

digestion of proteins by the toxin, with the liberation of abnormal amounts of non-protein nitrogen.

Cardiac and Voluntary Muscle. As the result of single or repeated lethal doses of toxin, fatal within four or five days, focal areas of necrosis may be found in the myocardium and in various voluntary muscles. Sometimes these are accompanied by small haemorrhages. Some of the necrotic fibres may be infiltrated with calcium at this time, and there may already be evidence of healing by the formation of young connective-tissue cells and fibres. The lesions are usually not accompanied by acute inflammation, although in some cases there may be collections of polymorphonuclear leukocytes about the necrotic fibres. Focal necroses do not always follow the injection of single minimal lethal doses, and smaller single or repeated doses may not produce them.

The Spleen and Liver. No destructive lesions are found in the spleen of rabbits with regularity, or even frequently, as a result of toxin alone. There is commonly an increase in iron pigmentation in the form of haemosiderin granules within phagocytic cells of the pulp. Occasionally, following single or repeated doses of toxin, focal haemorrhages alone or associated with necrosis of a few cells in the pulp, along with deposits of fibrinous and hyalinized exudate, are found. These commonly have a circular arrangement in the tissue, surrounding Malpighian bodies. They are regularly found in the spleens of rabbits dying from pneumococcal infection, and since they are not uncommon in the spleen of man in association with fatal pneumococcal infections, these lesions, in man and experimental animals, will be separately described in more detail.

The liver of the normal rabbit, unlike that of the dog, is not often apparently affected by pneumococcal toxin. The liver cells may have the appearance of cloudy swelling, and in experiments lasting for days the central liver cells may be somewhat atrophied. In these experiments, carried on for several years with many rabbits, *necrosis* of liver cells has rarely been found in *normal* rabbits following the injection of single or repeated lethal quantities of toxin.

Adipose Tissue. The injection of quantities of toxin capable of producing anatomical lesions is accompanied by a rapid loss of body-weight. There is, no doubt, some atrophy of most tissues, but the

atrophy and disappearance of all of the adipose tissues is very pronounced. In the gross the adipose tissue in the mesentery, about the kidneys and lumbar muscles, and wherever it can be found, becomes somewhat white and opaque. Eventually, thin veil-like membranes may be found representing the normal plump yellow masses of adipose tissue.

The Haemoglobin, Blood Cells, and Temperature. The effect of toxin on the haemoglobin and blood cells of 16 rabbits has been investigated. The animals used varied in weight from 1200 to 2480 grams. In one group single minimum lethal or larger doses were given. The other rabbits were given intravenous injections of toxin on four to seven successive days. At first sublethal doses of 0.5 cc. were injected, which were usually increased to minimal lethal or larger doses. The normal haemoglobin of these animals was 75 to 80 per cent (Talquist). The red blood cell counts varied from 4,776,000 to 9,976,000 per c.mm.

The single injections of lethal doses of toxin were usually fatal within five days. In these rabbits, on the day of death, the red blood cells were within normal limits. In some cases the haemoglobin had dropped to 70 per cent, the red blood cells to 4.5 million per cubic millimeter. The repeated daily doses of toxin produced considerable anaemia in some of the rabbits (Hgb. 55 to 60 per cent; red blood cells 2,964,000 to 3,952,000 per c.mm.). In others there was very little change (Hgb. 70 per cent; red blood cells 4,704,000 per c.mm.).

In general the toxin produces a moderate leukocytosis, 15 to 25,000 per c.mm., due chiefly to increase of the polymorphonuclear cells. In some animals the cells rise to 50,000 or 60,000 per cubic millimeter with repeated injections of toxin. The femur bone-marrow of these rabbits shows no hypoplasia, and is filled with blood-forming cells as in normal young rabbits. Some degree of oedema may be found, but the anaemia is apparently caused chiefly by haemolytic destruction of red blood cells in the circulation.

The temperature has been followed in many rabbits at frequent intervals—every three hours during the day and until midnight. The changes in temperature are quite regular. Following the first injection of toxin there is usually a rise of several degrees, 1° to 3°F., for several hours. The temperature then falls to normal or may be one or two degrees below normal for some days. If the animal dies there

is a gradual fall to a very low level, 96° to 98°F., for several hours before death. The effect of the toxin in causing subnormal temperature is very striking.

(*f*) *Antigenic Properties and the Duration of Immunity*

Although pneumococcal toxin (autolysate) is usually classified as an endotoxin, formed by lysis of the bacterial body, it contains nevertheless a true toxin, since it is actively antigenic. Parker (12) has reported the formation of antitoxin by repeated injection of toxin into horses.

In rabbits immunity to toxin, immunity to lethal doses of virulent Pneumococci, and antitoxin, all develop from the intravenous injection (1) of small nonpathogenic doses of toxin, increasing gradually in amount over a long period, (2) from the injection of small constant daily doses of toxin, at first slightly pathogenic, given over a short period of time (a week or more), and (3) from the injection of single or repeated sublethal pathogenic quantities of toxin. The passive transfer of antiserum produced by these three methods protects normal rabbits against the effects of toxin and against infection with pneumococci. In the following experiments the test of the degree of immunity to toxin, actively or passively acquired, is based on the presence or absence of albumin in the urine following the injection of toxin.

Antitoxin formed in rabbits from long immunization with increasing doses of toxin is much more potent against toxin than ordinary commercial antiserum. The serum of rabbits immunized over a period of seven months by the intravenous injection, at first of minute nonpathogenic doses of toxin, increasing in quantity from 0.005 cc. to 12 cc. doses, when injected intravenously into normal rabbits, neutralizes toxin injected a few minutes afterwards, in the proportion of 2 cc. of antitoxin to 1 cc. of toxin. Following the injection of toxin and antitoxin no albumin is excreted in the urine (Esbach). Five cubic-centimetres of commercial antiserum, Pneumococcus Type I, injected into a normal rabbit weighing 1800 grams, followed by the injection of 1 cc. of Pneumococcus Type I toxin may be followed within thirty hours by the excretion of 30 grams per liter of albumin (Esbach) in the urine.

The rabbits immunized by the first method described survive lethal

doses of virulent Pneumococci, 0.2 cc. of a 24-hour broth culture, when injected into the skin. The normal controls die within twenty-four hours. Five cubic-centimetres of serum of the actively immunized rabbit, given intravenously to a normal rabbit, infected intracutaneously immediately afterwards, protects the latter animal, which survives. The duration of the immunity actively acquired over a long period in the way described has not been tested.

When small constant quantities of toxin (0.2 or 0.3 cc.), at first pathogenic, are given intravenously every day in several injections to rabbits weighing from 1200 to 1500 grams, polyuria and slight albuminuria develop. The urine excreted in 24 hours may be double the normal volume, which is generally from 50 to 100 cc. The maximal degree of albuminuria is reached within the first 48 hours, and amounts at most to 3 or 4 grams per liter (Esbach). The albumin in the urine decreases rapidly thereafter, and by the eighth or tenth day none is excreted. For at least three months after the last injection the animal is immune to a single intravenous injection of toxin equal in volume to the total amount previously injected, and will survive lethal intracutaneous doses of virulent Pneumococci.

The serum of the rabbit actively immunized in this way contains antitoxin which can be passively transferred to protect a normal rabbit against at least one minimal lethal dose of toxin, and prevents the excretion of any albumin in the urine of the rabbit receiving the antitoxin. Five cubic centimetres of the serum of the actively immunized rabbit, injected intravenously into a normal rabbit, gives some protection against a lethal dose of virulent Pneumococci, for less inflammation is produced at the site of infection, without the usual extensive haemorrhage which occurs in the normal, unprotected controls, and the animal given antiserum lives two to three days longer than the control, which dies within twenty-four hours.

Lastly, active immunity against the effects of toxin rapidly develops in rabbits from the injection of one or two sublethal pathogenic doses, quantities which cause the excretion of large amounts of albumin in the urine, 20 to 30 grams per liter, and the lesions of "tubular" nephritis from which the animals may recover completely. The surviving rabbits are immune for at least two months to the reinjection of a single dose equal in amount to the original quantity given, and will

survive intracutaneous injections of lethal doses of virulent Pneumococci. The immunity to toxin acquired from the injection of sublethal pathogenic quantities of toxin gradually disappears, as illustrated by the following experiments.

The Loss of Immunity to Toxin Acquired from Sublethal Pathogenic Doses of Toxin

The following protocols illustrate the *rate* at which immunity disappears, immunity acquired from the injection of one or two sublethal pathogenic doses of toxin.

1. One week after recovery from antigenic, pathogenic doses. R. 56-25. Weight, 1540 grams. Given one injection of toxin of 2 cc., the minimal lethal dose being 3.08 cc. Urine: free from albumin on the sixth day. One week later the rabbit was bled from the heart for antiserum. Four days after bleeding, the injection of 3 cc. of toxin caused no albuminuria. The rabbit survived. Ten cubic centimetres of the serum of this rabbit protected a normal rabbit weighing 610 grams, against any albuminuria from the injection of 0.3 cc. of toxin. The control, a normal rabbit weighing 710 grams, was given 0.3 cc. of toxin and developed albuminuria for four days, amounting to 1.5 grams per liter within 24 hours.

2. Two months after recovery from antigenic, pathogenic doses. R. 56-15, weighing 560 grams. Given one dose of toxin of 0.56 cc. Excreted 20 grams of albumin per liter in the urine within 48 hours. Became oedematous on the third day. Urine was albumin-free by the sixth day and the animal recovered. Two months later, weighing 1200 grams, the injection of 1 cc. of the same toxin caused no albuminuria.

3. Four months after recovery from antigenic, pathogenic doses. R. 34–44. Weight, 1580 grams. Given one intravenous injection of 1 cc. of toxin. Excreted as much as 25 grams of albumin per liter in the urine. Urine contained no albumin on the sixth day. Four months later, weighing 2660 grams, the intravenous injection of 2 cc. of toxin caused the excretion of 35 grams of albumin per liter in the urine within 24 hours.

4. Thirteen and fourteen months after recovery from antigenic, pathogenic doses. R. 13–18. Weight, 1680 grams. In three days, given

3.5 cc. of toxin in two intravenous injections. The urine contained 18 grams of albumin per liter 24 hours after the first injection. Thirteen months after recovery, weighing 3220 grams, the intravenous injection of a single sublethal dose of 3.5 cc., the minimal lethal dose of this toxin being 6.4 cc., caused albuminuria, and death in six hours with symptoms of anaphylaxis (labored respiration and nasal discharge). The kidneys contained the lesions of "tubular" nephritis, and there were areas of fresh central and midzonal necrosis in the liver.

R. 13–12. Weight, 1730 grams. Survived a single injection of 3.4 cc. of toxin, one minimal lethal dose. The urine contained 20 grams of albumin per liter within 48 hours. Fourteen months later, weight 3680 grams, one intravenous injection of 3.4 cc. of toxin was given, half of the minimal lethal dose. A few hours afterwards the signs of anaphylaxis were present. There was difficult, wheezing respiration, with mucoid nasal discharge, and the animal died within 19 hours. No urine was excreted. At autopsy the urine in the bladder contained five grams of albumin per liter. Microscopically, the kidneys showed the changes of diffuse "tubular" nephritis, with necrosis of epithelium, and in the liver there were areas of fresh necrosis of central and midzonal liver cells.

In *summary*, immunity against the effects of the toxin, and protection against infection is developed, along with the formation of strong antitoxin which may be passively transferred, from the repeated injection of *non-pathogenic* doses increasing in amount. The antitoxin formed in this way, over a period of seven months, may neutralize toxin in the proportion of two to one.

From the injection of single or a few repeated *sublethal pathogenic* doses of toxin, "tubular" nephritis with massive albuminuria is produced, coördinately with the development of active immunity and the formation of small amounts of antitoxin which may be passively transferred. Large quantities of such antiserum protect normal animals against the effect of small pathogenic doses of toxin. The immunity developed as a result of these sublethal pathogenic doses gradually disappears. It is distinctly decreased after four months, and at the end of a year the reinjection of a single dose equal to or less than the original immunizing dose may cause death of the animal, with signs

of anaphylaxis, in addition to fresh necrosis of central and midzonal liver cells, necrosis of glomerular and tubular epithelium and albuminuria.

2. EXPERIMENTAL PNEUMOCOCCAL NEPHRITIS AND NEPHROSIS

It has already been pointed out that in the human being the differences in the anatomical and functional disorders of the kidney in nephrosis, tubular nephritis, and in acute and chronic glomerulonephritis are chiefly differences in degree, not in kind. The fundamental similarity in the nature of the physiological and histological processes occurring in these various forms of nephritis is further illustrated by the following description of experimental pneumococcal nephritis and nephrosis. Before each form of experimental nephritis is described in detail particular points which the experiments illustrate may be summed up as follows: (1) With a single poison, pneumococcal toxin, forms of nephritis corresponding anatomically and physiologically to nephrosis, tubular nephritis, and to glomerulonephritis may be reproduced experimentally, depending on the dosage of toxin and the duration of administration. (2) By means of a single dose of toxin tubular nephritis may be reproduced, characterized by anuria or oliguria, albuminuria, slight haematuria, casts in the urine, nitrogen retention, lipaemia, low plasma proteins, and oedema. Histologically, necrosis, calcification, and regeneration of epithelium are the chief demonstrable lesions. If recovery takes place, the function of the kidney and the histological structure return to normal. (3) Repeated doses of toxin, equal to or somewhat smaller than the single dose used to produce tubular nephritis, given in several daily injections, cause changes in the urine and blood chemistry like those just enumerated, differing from them quantitatively, however. Lesions are found in the kidneys like those produced by single doses of toxin, with, in addition, the presence of more definite evidence of damaged glomerular capillaries, *i.e.*, more numerous haemorrhages, and clots of fibrinous exudate within the glomerular capsule and between capillary tufts. (4) Repeated pathogenic doses of toxin given over a longer period, at first in amounts smaller than minimal lethal doses, cause albuminuria, haematuria, lipaemia, and oedema; and, depending on the dosage of toxin, are accompanied either by the development of complete immunity to the effects

of the doses of toxin used and recovery, or by the development of the clinical and anatomical features of progressive glomerulonephritis. (5) By means of very much smaller doses of toxin nephrosis is reproduced, characterized by oliguria, albuminuria, slight microscopical haematuria, casts in the urine, lipaemia, diminishing plasma protein, normal non-protein nitrogen in the blood, and oedema. The histological lesions in the kidney consist chiefly of swelling of the epithelial cells, and of other changes indicative of some damage, with very little actual necrosis of cells. The disease may be continued until immunity to the doses of toxin used develops. After the disappearance of immunity actively acquired, the kidney is again susceptible to repeated damage by the same toxin. (6) In any form of nephritis, whether corresponding clinically and anatomically to nephrosis, tubular nephritis, or to acute and chronic glomerulonephritis, there is but little correlation between the fundamental functional changes in the kidney and the demonstrable histological lesions. No functional disorder can with certainty be ascribed to necrosis alone of tubular and glomerular epithelium. No visible lesions can so far assuredly account for anuria or oliguria, the most fundamental disorders in the function of the kidney. The changes in the glomerular capillaries responsible for the excretion of albumin, globulins, and of red blood cells are not recognizable by ordinary histological methods. The glomerular changes which are demonstrable, (regeneration of tissue, organization of exudate, and thrombi in glomerular capillaries), like the functional disorders (albuminuria and haematuria), are both *results* of damage. In progressive nephritis, diminishing necrosis of cells, and in part decreasing albuminuria are expressions of increasing immunity to the effect of toxin. The obstruction of glomerular capillaries by thrombi, and by clots of exudate and scar-tissue formation in the glomerular capsules, probably accounts for the inability of the kidney to excrete waste products, especially non-protein nitrogen, and may account to some extent for the decreasing albuminuria. The fact that haemorrhages from the glomerular capillaries may continue to occur in progressive nephritis, when albuminuria is decreasing, would suggest that the mechanism responsible for bleeding from the capillaries at this stage may be different from that which causes the escape of fluid containing albumin and globulins of the blood plasma. Indeed, it seems obvious that

haemorrhages are caused by the rupture of *scattered individual* capillaries from time to time. This may not necessarily lead to massive albuminuria, which is probably the result of the constant escape of albumin through intact, patent, but damaged glomerular capillaries *throughout* the kidney. (7) The percentage of albumin in the ascitic fluid formed in association with nephritis, produced by relatively large repeated doses of toxin, is higher than that of albumin found in the ascitic fluid of nephrosis caused by smaller doses of toxin. The smaller percentage of albumin in the ascitic fluid of experimental nephrosis is to be regarded as the result of less severe damage to the tissue capillaries than that which occurs in experimental nephritis.

(*a*) *Acute Tubular Nephritis*

The method used in producing this form of nephritis, and the progressive changes in the urine, from anuria to complete recovery to normal in many instances, have been described with the discussion of the effects of the toxin on the tissues in general. Table I contains the data concerning dosage of toxin and the urinary and blood chemical changes found in experimental "tubular" nephritis. It may be said further that, in the kidneys of animals dying late in the course of tubular nephritis, when the urinary function is returning to normal, widespread calcification of the necrotic epithelium may be found. In this respect also the experimental disease resembles "tubular" nephritis in the human being, such as that caused by bichloride of mercury.

(*b*) *Acute and Progressive Glomerulonephritis*

The production of pneumococcal nephritis with oedema in rabbits, and the changes in the kidneys have already been reported (13). In these experiments several daily intravenous injections were used, somewhat smaller than minimal lethal doses of toxin. Recently such experiments have been repeated, and in a few animals the disease has been continued until clinical symptoms and renal lesions simulating progressive glomerulonephritis in man have appeared. The quantities of toxin given and the results obtained in experimental acute and progressive glomerulonephritis, together with the changes in the blood chemistry and urine, are shown in table III. The functional disorders of the kidney, and the changes in the proteins, non-protein nitrogen

TABLE III

Acute and progressive glomerulonephritis

EXPERIMENT NUMBER	WEIGHT OF RABBIT	DURATION OF EXPERIMENT	DOSAGE OF TOXIN		BLOOD CHEMISTRY AT THE END OF THE EXPERIMENT						FLUIDS		ALBUMINURIA		FLUID IN TISSUES AND BODY CAVITIES		
			Number of doses	Total quantity given	N.P.N.	Cholesterol	Plasma protein	Albumin nitrogen	Globulin nitrogen	A/G ratio	Total volume of fluid injected	Total volume of urine excreted	Grams per liter (average, Esbach)	Total albumin excreted	Oedema	Hydrothorax	Ascites
	grams			*cc.*	*mgm. %*	*mgm. %*	*grams %*	*grams %*	*grams %*		*cc.*	*cc.*		*grams*			
12-5	1,650	26 hrs.	2	3.0	140	82	3.89	0.265	0.358	42/58	0	23	10.20	0.234	0	0	0
51-5	5,500	2 days	3	5.0	110	194	4.25	0.329	0.351	48/52	60	70	6.0	0.420	0	0	0
55-1	1,240	2½ days	3	2.5	260	230	3.16	0.277	0.228	55/45	0	16	14.08	0.224	++++	4 cc.	5 cc.; albumin, 1.1%
12-3	2,150	3 days	3	4.0	240	143	4.37	0.292	0.408	42/58	0	163	8.20	1.336	0	0	0
12-2	1,640	5 days	5	7.0	300	385	5.37	0.316	0.544	37/63	0	519	6.25	3.217	++++	0	0
55-2	1,300	5 days	5	5.5	106	333	3.52	0.276	0.288	49/51	0	264	11.18	2.930	++++	0	0
34-52	1,320	9 days	8	4.4	100	39	2.87	0.226	0.234	49/51	348	633	3.62	2.278	++++	0	12 cc.; albumin, 0.15%
34-15	1,520	15 days	20	25.8	285	136	4.55	0.395	0.333	54/46	15	1134	2.70	3.061	0	0	12 cc.; albumin, 1.0%

and cholesterol of the blood are similar in kind to those caused by single doses of toxin used in the production of "tubular" nephritis. The repeated doses, however, lead to the formation of clots within the glomerular capsules, containing fibrin, red blood cells and leukocytes. These clots, quite apart from the damage to the glomerular capillaries caused by the toxin, further interfere with the function of the kidney by obstructing the outlet from the glomerular capsule at the neck of the proximal convoluted tubule. Excessive regeneration of glomerular epithelium occurs also, as in chronic glomerulonephritis in man.

If repeated injections of toxin are given over a long period, at first in sublethal amounts, and are subsequently increased, or if daily doses, more or less constant in quantity, are supplemented at intervals by occasional larger doses as are necessary to maintain a considerable degree of albuminuria, two distinct effects may be observed. (1) Considerable immunity to the toxin develops, and (2) in the kidney the lesions of diffuse progressive glomerulonephritis develop, accompanied by nitrogen retention in the blood. The glomerular lesions resemble those found in the so-called nephrotic stage of chronic glomerulonephritis.

Immunity manifests itself physiologically and anatomically. Albuminuria and lipaemia decrease, and the plasma proteins and albumin-globulin ratio return toward or to normal. A quantity of toxin injected several days after the beginning of the experiment may increase the amount of albumin excreted during the following twenty-four hours above the amount excreted on the previous day, but this dose, which may be larger than a single minimal lethal dose for this animal, is not followed by the albuminuria which the same dose causes in a normal animal of the same weight. The decreasing degree of cholesterolaemia also apparently indicates that the toxin is being neutralized. While the plasma cholesterol of these animals may be above normal, and some albuminuria and haematuria are still present, the elevation of the cholesterol and the degree of albuminuria are not commensurate with the quantity of toxin given. Furthermore, both the cholesterolaemia and albuminuria of rabbits with this progressive form of nephritis (34, R. 52 and 15, table III) are much less marked than in rabbits with acute nephritis produced by means of much smaller quantities of toxin (tables I and III).

There is also very definite histological evidence of some immunity to the effect of the toxin on the tissues. Microscopically, in the tissues of rabbits with the progressive form of nephritis, produced by repeated doses of toxin, progressive necrosis of cells is not found in the organs in which, ordinarily, necrosis occurs following the injection of single lethal doses into normal rabbits without any immunity. That is, there is not widespread necrosis of renal epithelium, and increasing foci of necrosis in the myocardium and voluntary muscles are not found. One may find healing and scarring at the site of previous necrosis produced by the early doses which were given. There may be cellular focal scars in the myocardium and voluntary muscles, sometimes with deposits of calcium. In the kidneys regenerated epithelium is found, and some necrosis of tissue continues, but it is far out of proportion to the quantity of toxin given.

The clinical course of the progressive nephritis may be summarized briefly. Varying degrees of oliguria and albuminuria developed at first in each rabbit. The albumin excreted in the urine varied from 18 to 0.5 gram per liter, decreasing with the progress of the disease. The volume of urine increased after the initial period of oliguria. The urinary sediment contained casts and a few leukocytes. At times no red blood cells were found in the urine, but they were often present in small numbers. Gross haematuria occurred once in the course of one experiment (34, R. 52).

Oedema of the subcutaneous tissues about the sacrum and of the thighs appeared on the third day of the disease in one of the rabbits. In the other (34, R. 15) no oedema was ever demonstrated in the subcutaneous tissues. At autopsy, however, there were 12 cc. of ascitic fluid, containing 1 per cent of albumin (Esbach).

In these experiments elevation of the non-protein nitrogen of the blood is associated with excessive regeneration of glomerular epithelium and organization of exudate within the glomerular capsules. During the longest experiment (34, R. 15), fifteen days in duration, 25.8 cc. of toxin were injected. For four days before the end of the experiment daily doses of toxin were given, each of 2 to 3.6 cc. During this time the albumin in the urine decreased from 5 to 1.5 grams per liter. At the end of the experiment the plasma protein and A/G ratio were within normal limits. The blood cholesterol was only 136 mgm. per

cent, an elevation which may be produced within twenty-four hours in a normal animal by a single injection of 1 cc. of toxin. The non-protein nitrogen of the blood, however, was very high, 285 mgm. per cent. Since cholesterolaemia continued to diminish, along with albuminuria and necrosis of tissue, as immunity increased, despite the rising non-protein nitrogen of the blood, these data suggest that the rise in plasma cholesterol which occurs in normal non-immune animals is related to abnormal development of cholesterol from the action of toxin on the tissues.

The lesions in the kidneys of the animals with progressive glomerulonephritis are numerous and diffuse, and the changes found are especially processes of healing and repair. Some necrosis of epithelial cells of tubules and glomeruli is found, but it is not widespread. Many of the tubules in the cortex are lined with flat regenerated epithelial cells which stain deeply, and in some cells mitotic figures are found. Within some tubules there are hyaline casts, composed of necrotic cell débris fused together. Droplets of neutral fat are present within epithelial cells of some of the glomeruli and tubules. No doubly refractile fluid crystals, showing Maltese crosses when cooled after heating, have been found, either in the urine of the rabbits or in smears and frozen sections of the kidneys.

Many of the glomeruli are much larger than normal. The capillary tufts form conspicuous, compact, cellular masses, separated from each other by distinct clefts. The individual capillaries in each tuft may appear fused together, and are often difficult to see distinctly. The regenerated epithelial cells over the tufts are large and plainly visible. The nuclei of some cells show mitotic figures. There are more numerous epithelial cells over the tufts than normal, and in places solid masses of cells are found filling the clefts between adjacent tufts of capillaries. Occasionally, there are solid cellular masses occupying portions of the capsular spaces. In many places cellular adhesions are found between regenerated cells of the visceral and parietal layers of the glomerular epithelium. Between many tufts of capillaries, and in the outer part of the capsular spaces, there are coagulated masses composed of hyaline exudate and nuclear fragments, containing also a few red blood cells occasionally. These masses of exudate in the capsules have a crescent-like form, and are often partly organized

by cells surrounding and growing within them. It is well-known that, in general, healing processes often develop to an extent greater than necessary for the actual repair of damaged tissue, and under these circumstances usually lead to interference with normal function (14). The glomerular changes just described seem sufficient to account for the retention of non-protein nitrogen in the blood, and may in part account for the decreased amount of albumin excreted in the urine. The latter, however, seems chiefly dependent on the progressive development of immunity, and that this is the chief mechanism responsible is illustrated by the experiments in which both albuminuria and non-protein nitrogen of the blood decreased in the absence of destructive glomerular lesions, while almost complete immunity to increasing doses of toxin eventually developed (table II).

(*c*) *Experimental Pneumococcal Nephrosis in Rabbits*

(*c1*) *Methods and Material*

The minimal lethal dose of the toxin used in the following experiments was 0.2 cc. per 100 grams weight of rabbit. Most of the experiments were carried out on young white rabbits varying in weight from 1020 to 1560 grams.

The rabbits were kept in individual cages with wire-screen bottoms over funnel-shaped pans. The cages were cleaned once or twice daily as necessary. Gauze caps were fastened over the funnels of the cages, and the urine was filtered through these as excreted and collected in glass bottles. The urine was always collected twice each day, in the morning and at night, and sometimes more frequently. Albumin was measured in Esbach tubes.

At the end of each experiment the animal was bled from the heart, killed and autopsied. The blood was collected with dry sterile syringes, and placed in bottles containing dried potassium oxalate (8 drops of 3 per cent oxalate for 10 cc. of blood) to prevent clotting.

The non-protein nitrogen of the whole blood was determined by the method of Folin and Wu, the total protein of the plasma by the Kjeldahl method, and the plasma cholesterol was measured colorimetrically (Bloor). Table IV shows the blood chemistry of sixteen normal control rabbits, determined by the same methods. Table V contains the details of each experiment. In the first three experiments tabu-

lated (R. 27, R. 28 and R. 25) the rabbits were bled and killed 24, 48, and 72 hours, respectively, after a single dose of toxin in order to determine what changes had taken place in the blood proteins, cholesterol, and non-protein nitrogen at these intervals.[1]

In order to produce nephrosis in rabbits, small quantities of toxin were injected intravenously at various intervals so regulated by trial and error that there was continuous excretion of albumin in the urine without the production of necrosis of tissue and the lesions of "nephri-

TABLE IV

Chemical determinations on the heart's blood of normal rabbits

NUMBER OF ANIMAL	N.P.N.	CHOLESTEROL	PLASMA PROTEINS	ALBUMIN NITROGEN	GLOBULIN NITROGEN	A/G RATIO
	mgm. %	*mgm. %*	*grams %*	*grams %*	*grams %*	
B-1	39	62	5.64	0.597	0.305	66/34
B-2	36	71	5.77	0.583	0.341	63/37
R-2	43	87	4.79	0.572	0.195	74/26
R-94	36	55	4.71	0.421	0.333	56/44
34-2	36	65	4.77	0.522	0.242	68/32
34-13	36	91	4.85	0.549	0.227	71/29
34-16	37	67	5.24	0.592	0.247	71/29
34-22	51	75	5.39	0.579	0.283	67/33
*34-18	50	53	5.17	0.551	0.275	67/33
*34-26	31	68	5.29	0.614	0.232	72/28
56-17	36	50	5.27	0.514	0.330	61/39
56-18	34	33	4.99	0.594	0.205	74/26
56-19	41	31	5.44	0.581	0.290	67/33
56-20 A	33	43	4.79	0.526	0.241	68/32
56-20 B	36	36	5.40	0.513	0.351	59/41
R-95	41	67	4.53	0.431	0.294	59/41

* These two rabbits were given 500 cc. of normal saline solution, injected intravenously, over a period of four days preceding the blood chemical determinations.

tis" in the glomeruli, *i.e.*, thrombi in the capillaries and clots of exudate in the capsular spaces. To prevent anuria or very marked oliguria and elevation of the non-protein nitrogen of the blood, most of the rabbits were given several daily intravenous injections of 5 per cent glucose or normal saline solution. Usually the animals were given

[1] The methods described here for collection of urine and determination of blood chemistry were similarly employed in all of the experiments described in the preceding sections.

TABLE V

Experimental nephrosis

EXPERIMENT NUMBER	WEIGHT OF RABBIT	DURATION OF EXPERIMENT	DOSAGE OF TOXIN		BLOOD CHEMISTRY AT THE END OF THE EXPERIMENT						FLUIDS		ALBUMINURIA		FLUID IN TISSUES AND BODY CAVITIES		
			Number of doses	Total quantity given	N.P.N.	Cholesterol	Plasma protein	Albumin nitrogen	Globulin nitrogen	A/G ratio	Total volume of fluid injected	Total volume of urine excreted	Grams per liter (average, Esbach)	Total albumin excreted	Oedema	Hydrothorax	Ascites
	grams			*cc.*	*mgm. %*	*mgm. %*	*grams %*	*grams %*	*grams %*		*cc.*	*cc.*		*grams*			
34-27	1,340	24 hrs.	1	0.3	34	150	4.72	0.558	0.198	74/26	65	128	2.25	0.256	0	0	5 cc.; albumin, 0.6%
34-28	1,240	48 hrs.	1	0.3	34	130	4.13	0.453	0.208	68/32	117	159	2.83	0.449	0	0	0
34-25	1,020	72 hrs.	1	0.45	30	154	3.56	0.316	0.257	55/45	234	270	3.83	1.034	+	6 cc.	25 cc.; albumin, 0.2%
34-7	1,420	4 days	3	1.4	31	250	3.71	0.245	0.349	41/59	247	243	6.75	1.371	0	4 cc.	10 cc.; albumin, 0.2%
34-11	1,440	4 days	3	1.5	57	187	3.02	0.215	0.268	44/56	247	379	7.12	1.994	++++	0	2-3 cc.
34-20	1,200	4 days	3	1.4	41	158	2.84	0.322	0.132	71/29	260	225	3.82	0.859	0	+	20 cc.; albumin, 0.15%
34-21	1,140	4 days	2	0.9	52	280	3.67	0.272	0.312	47/53	260	498	2.87	1.429	++	8 cc.	20 cc.; albumin, 0.2%
34-14	1,340	5 days	3	1.2	57	250	4.34	0.393	0.302	56/44	288	406	1.52	0.617	0	0	0
34-31	1,270	5 days	11	2.9	36	160	3.59	0.329	0.245	57/43	234	594	1.25	0.742	+	0	0
34-50	1,240	5 days	4	3.5	31	164	3.48	0.216	0.341	39/61	275	777	1.52	1.181	+	+	8 cc.; albumin, 0.20%
56-1	1,560	5½ days	10	3.0	40	158	3.09	0.228	0.266	46/54	192	497	3.58	1.779	+	+	30 cc.; albumin, 0.15%
34-23	1,150	6 days	11	4.0	50	333	3.77	0.294	0.310	48/52	25	935	2.91	2.720	++++	0	0
34-40	3,070	3 days	6	10.0	47	146	4.59	0.349	0.385	48/52	80	552	0.95	0.541	0		Living—well.
61-1	3,100	6½ days	5	3.5	38	125	5.85	0.353	0.583	38/62	460	740	2.90	2.146	0	2 cc.	4 cc.; albumin, 0.55%

three injections of toxin, each of 0.2 to 0.6 cc., together with daily injections of fluid. In one rabbit (R. 23) ten small doses of toxin of 0.3 cc. each, and one dose of 1 cc., caused polyuria, and it was therefore unnecessary to give fluid to this animal. The injection of glucose or saline alone into normal rabbits causes no albuminuria, nor are there any changes in the proteins, non-protein nitrogen, and cholesterol of the blood. The blood chemistry determinations of two of these controls are included in table IV (R. 34–18 and R. 34–26).

(*c2*) *Duration*

Most of the experiments lasted from four to six days, and were terminated when the diminishing excretion of albumin in the urine indicated that immunity was developing from the doses of toxin used. It has already been shown that the immunity gradually disappears, and it is apparent from the last two experiments tabulated in table V (R. 34–40 and R. 61–1) that the production of nephrosis may be repeated in animals after immunity disappears. To this extent at least the experimental disease simulates the remissions and exacerbations of chronic nephrosis in the human being.

One of these animals, R. 34–40, was given small daily doses of toxin and several larger doses of 1 cc. each over a period of two weeks, from which there was complete recovery. During the experiment albumin was excreted in the urine daily, from 0.5 to 30 grams per liter, and oedema developed. No blood chemical determinations were made. Four months after the urine was normal, when some degree of immunity was still present, the injection of a total of 10 cc. of toxin, given in two daily doses for three days, caused the excretion of one to two grams of albumin per liter in the urine. There was no demonstrable oedema. At the end of the third day blood was withdrawn from the heart for chemical determinations. The non-protein nitrogen was normal, the cholesterol elevated, the albumin of the plasma was low and the albumin-globulin ratio inverted. This animal recovered again.

The second rabbit, R. 61–1, was first used in experiments testing the strength of antitoxin. In five days it received 8.5 cc. of toxin given in three doses, together with a small amount of weak antiserum. On the third day the urine contained 33 grams of albumin per liter. The albuminuria disappeared and the animal recovered completely. One year and a half later, weighing 3100 grams, the rabbit was given 3.5

cc. of toxin in five doses. On the second day the urine contained 28 grams of albumin per liter. The albuminuria decreased to 0.25 gram per liter on the sixth day. Oedema was never demonstrable. The rabbit was killed. The pleural and abdominal cavities contained small amounts of fluid. The ascitic fluid contained 0.55 per cent of albumin. The non-protein nitrogen of the blood was normal, the cholesterol elevated, and the albumin fraction of the plasma protein decreased, with inversion of the albumin-globulin ratio. The kidneys of this rabbit show a few scattered focal scars, with completely obliterated glomeruli. These lesions were probably caused by the toxin given a year and a half before. The recent doses produced changes like those to be described in the kidneys of the other rabbits with experimental nephrosis.

(*c3*) *The Urine*

The volume of urine excreted during the experiments varies from day to day, increasing in amount as the percentage of albumin diminishes. At first there is definite oliguria in most of the animals, when the urine may contain as much as 16 grams of albumin per liter, or more, depending chiefly on the quantity of toxin given. As the experiments progress the excretion of albumin decreases, usually to half a gram per liter at the end. The total volume of the urine and the amount of albumin excreted by each rabbit during the experiments are shown in table V.

During the periods of oliguria the urine is dark brown in color. The specific gravity is high, 1.020 to 1.030 or higher. Fresh specimens are slightly acid or neutral to litmus paper, but quickly become alkaline.

Microscopical examinations of centrifugalized specimens show at first no casts, or moderate numbers of large granular casts. These may become very numerous, and later on hyaline casts appear. In the urine of some of the animals few or moderate numbers of red blood cells may be found at times.

(*c4*) *The Non-Protein Nitrogen and Cholesterol of the Blood*

The non-protein nitrogen of the blood of the animals with experimental nephrosis varied from 30 to 57 mgm. per cent. The minimum and maximum values obtained in sixteen normal control rabbits were 31 and 51 mgm. per cent (table IV).

It is to be emphasized here that large quantities of fluid were given intravenously to some of the rabbits, 60 to 70 cc. daily, in order to prevent anuria, although the disease can be produced without the injection of fluid in animals which naturally develop polyuria, as in one animal (R. 34–23) given only 25 cc. of normal saline on the first day of the experiment, six days in duration. The prevention of anuria is apparently the mechanism responsible for the absence of high blood non-protein nitrogen. The latter cannot be explained as the result of dilution of the blood by the fluid injected, as the elevation of blood cholesterol and inversion of the A/G ratio indicate. Furthermore, the intravenous injection of similar quantities of fluid into normal rabbits causes no demonstrable dilution of the non-protein nitrogen, cholesterol, or protein of the blood. The last injection of fluid was given to the controls (table IV) and to the rabbits with nephrosis from ten to twelve hours before blood was withdrawn for chemical analysis.

The non-protein nitrogen of the blood was determined only once in each animal, at the end of the experiment, and may have been elevated earlier in the disease. Since the figures are practically normal at the end of each experiment, although repeated doses of toxin were given, the last usually within less than twenty-four hours before blood was taken for chemical analysis, and since elevation of the non-protein nitrogen occurs during certain stages of the disease in man, the experimental disease seems analogous to nephrosis in the human being as regards the non-protein nitrogen of the blood, as in other respects.

It is not necessary to comment here on the elevation of blood cholesterol in experimental pneumococcal nephrosis, since the mechanism responsible for it is no doubt the same in both experimental nephrosis and nephritis. It may be seen from table V, however, that the cholesterolaemia cannot be explained in these experiments as the result of low plasma protein, an explanation sometimes offered, since it may be present within twenty-four hours after the initial dose of toxin is injected, before the plasma proteins are appreciably altered.

(*c5*) *Plasma Proteins and Oedema*

In rabbits with nephrosis characteristic decrease in the protein content of the blood plasma takes place and is accompanied by marked alteration or complete inversion of the albumin-globulin ratio. The

gradual development of the change is shown by the determinations made at the end of 24, 48, and 72 hours, respectively, following single doses of toxin. Within 72 hours the total plasma protein may be quite low (3.56 grams per cent), and oedema, ascites, and hydrothorax may all be present (R. 34–25, table V). The animal killed 48 hours after the beginning of the experiment (R. 34–28) developed neither demonstrable oedema nor ascites within this period. In one other rabbit, R. 34–14, there was no demonstrable oedema during the experiment, five days in duration, and at autopsy there was no ascites or hydrothorax. In the others there was either oedema during the experiment, or fluid was found in the body cavities at autopsy, or both were present. The oedema, when it occurred, usually appeared on the third day and increased in amount.

(*c6*) *The Kidneys and Other Tissues*

The kidneys of the rabbits with nephrosis are not precisely alike in every instance. Grossly, they are alike in all of the experiments in showing some degree of enlargement and pallor. In no case are they greatly enlarged, or very yellow, as in nephrosis in the human being, since the kidneys of the rabbits contain relatively little fat. The surfaces are smooth, without any scars. The kidneys of one rabbit showed a number of minute scattered haemorrhages on the surface. This rabbit was infected with Pneumococci during the course of the nephrosis, and developed septicaemia and peritonitis. No haemorrhages were seen in the gross in the kidneys of the other rabbits.

The microscopical changes are similar in all of the kidneys, but differ somewhat in degree. The characteristic lesions found may be briefly enumerated: (1) The changes which are demonstrable by histological methods are found only in the epithelium of the glomeruli and tubules. (2) No alterations in the structure of the glomerular capillaries have been recognized. (3) In none of the kidneys are fresh fibrinous or organized exudates found in the glomerular capsule or tubules, and there is no evidence of hyalinization of capillary tufts, or occlusion of individual capillaries by endothelial or other cells. In the kidneys of three rabbits (R. 20, 23 and 31, table V), excluding the one with septicaemia, a few widely scattered haemorrhages are found in the tubules. (4) The changes in the glomerular and tubular epi-

thelium consist chiefly of cloudy swelling of the cytoplasm and enlargement of the nuclei, which occasionally show mitotic figures. Some cells contain "colloid" droplets of cytoplasm, and some are desquamated into tubules and glomerular capsules. In some of the kidneys there are a few scattered necrotic epithelial cells. In most, however, there is no definite necrosis of cells, and no calcified débris is found in any case. (5) With increasing duration, dilatation of some convoluted tubules becomes more conspicuous, and some of the wide tubules contain casts. The capsular spaces of the glomeruli generally show dilatation similar to that of the convoluted tubules. Some of the widened tubules are lined with flat epithelial cells, evidently regenerated. (6) In the glomeruli of some of the kidneys there are conspicuous epithelial cells with large clear nuclei over some of the capillaries, and these occasionally form points of adhesion between capillary tufts and Bowman's capsule. (7) In the kidneys of the animals killed at the end of 72 hours, or later, moderate numbers of globules of neutral fat are found in the epithelium of some glomeruli and convoluted tubules, and in the cells of Henle's loop in a few cases. (8) No doubly refractile fluid lipoid crystals have been found either in the kidneys or in the urine of these rabbits. (9) The arterioles in the kidneys show no lesions.

It may be said here that the kidneys of rabbits which have recovered from experimental nephrosis contain no diffuse lesions of the glomeruli or tubules; these are histologically normal. Focal scars may be found in small numbers.

Single microscopical sections were made in each case of the heart, lung, liver, spleen, adrenal, femur bone-marrow, and of one or two voluntary muscles. The last were usually taken from the lumbar and gluteal regions. The sections of the heart were made transversely through the walls of both ventricles, at the base of the heart, and generally included some of the valves and papillary muscles. In a few cases sections were made of the aorta, thyroid gland, and intestine. The following lesions are found in these tissues.

In the section of one heart (R. 34-27), at the end of 24 hours, there are a few small foci of necrosis. In five other cases there are small focal cellular scars in the myocardium, associated in two cases with minute areas of haemorrhage. The voluntary muscle of two of the rabbits contains small focal areas of calcified necrotic fibers.

In the lung of one rabbit a few small organizing haemorrhages are found, and there is marked oedema of the submucosa and serosa of the large intestine, especially of the appendix, of two rabbits. The individual liver cells may have the appearance of cloudy swelling, and there is usually slight atrophy of the central and midzonal liver cells.

(*c7*) *The Treatment of Experimental Nephrosis with Antitoxin*

A few experiments have been carried out in the attempt to determine the effect of intravenous injections of antitoxin on the course of experimental nephrosis in rabbits. In these experiments, after the appearance of well-marked albuminuria, daily doses of antitoxin were given, while also each day the injection of a small quantity of toxin was repeated. Large quantities of ordinary commercial antiserum, 15 to 45 cc. daily, did not appear to affect the course of the disease in any way. It seems probable, however, that antitoxin obtained from animals immunized over a long period with repeated doses of toxin may be more effective. At the present time further experiments are being carried out, using concentrated antiserum, including that obtained from the immunization of animals by means of increasing quantities of pneumococcal toxin.

3. COMPARISON BETWEEN THE EFFECT OF PNEUMOCOCCAL TOXIN ON THE TISSUES OF MAN AND ANIMALS

Most of the lesions produced experimentally in animals by means of the toxin are known to occur at times in man during pneumococcal infections. Widespread purpura, which is readily produced by the injection of toxin into dogs and mice, but is not seen in rabbits, occurs rarely in man with pneumococcal infections (15). Haemorrhages in the spleen of man during fatal pneumococcal infections are quite common, however. These lesions, it seems, have not been described. They are readily reproduced in dogs and rabbits infected with pneumococci, and in dogs by means of the toxin. These experiments will be reported in more detail separately.

Focal necrosis of voluntary muscle, occasionally accompanied by haemorrhage, has been described as occurring during the course of lobar pneumonia in man (16). The voluntary muscles of rabbits are easily damaged by the toxin, while in dogs the opposite is true.

It is well-known that jaundice is sometimes seen with pneumococcal lobar pneumonia. The mechanism responsible for it has been discussed by Rich (17), and the evidence indicates that jaundice is the result of damage to the liver cells from bacterial toxin and anoxaemia together with excessive destruction of red blood cells. The toxin alone is able to cause both haemolysis of red blood cells and necrosis of liver cells. The red blood cells and liver cells of dogs are extremely sensitive to the toxin, while the blood cells of normal rabbits are much less susceptible to damage. Histologically, the liver cells of normal rabbits, with rare exception, are apparently unaffected.

Concerning the occurrence of pneumococcal nephrosis and nephritis in man, the cases described previously (1), and the experimental production of nephrosis with pneumococcal toxin included here, suggest that some examples of lipoid nephrosis are caused by chronic pneumococcal infection. Acute pneumococcal nephritis, as recently described (18), with diffuse lesions of the glomeruli and tubules, occurs in a considerable proportion of infants and children with extensive fatal pneumococcal infections. Since also more chronic forms of the disease may be experimentally reproduced by the use of toxin, it seems possible that certain examples of subacute and chronic glomerulonephritis in man may be associated in etiology with pneumococcal infection.

4. DISCUSSION AND CONCLUSIONS

(*a*) *The Relation Between Nephrosis and Nephritis*

It appears from the clinical and histological studies of the examples of lipoid nephrosis described heretofore (1), and from the above experiments as well, that lipoid nephrosis and glomerulonephritis are fundamentally alike in the nature of their clinical expressions. Each is characterized by some degree of albuminuria and globulinuria, haematuria, lipaemia, and anaemia. The oedema of both is similar in pathogenesis, at least in so far as the latter is explicable in either disease.

The progressive loss of the power of the kidney to excrete nitrogen waste in glomerulonephritis constitutes the one essential clinical difference between these two forms of Bright's disease. This fundamental clinical distinction depends on the one basic histological difference between the kidneys in nephritis and nephrosis, namely, the progressive destruction of the majority of the glomeruli by healing processes in

glomerulonephritis, *i.e.*, organization of clots within the glomerular capsules, and excessive regeneration of glomerular epithelium. The usual explanation given, that nephrosis is a "degenerative" disease of the tubules, and that glomerulonephritis is an inflammatory disease of the glomeruli is confusing, and adds nothing towards an explanation of the pathogenesis of any single manifestation of lipoid nephrosis, while at the same time this classification obscures the fact that so-called degenerative changes in the epithelium of the kidney tubules are found not only in nephrosis but also in the tubules in glomerulonephritis, as well as in the glomerular epithelium in both diseases. Furthermore, the lesions supposed to be specific for glomerulonephritis also occur to a slight extent in nephrosis, as evidenced by the slight microscopical haematuria, by the presence of occasional adhesions formed by regenerating glomerular epithelium (the epithelial crescents of glomerulonephritis differ only in size and number of cells from the isolated regenerating epithelial cells found here and there in the glomeruli in nephrosis), and manifested by the presence of the isolated focal glomerular scars already described (1). Indeed, in glomerulonephritis there is even more "degeneration" of tissue (actual necrosis of cells) than in nephrosis, and there is often equally abundant doubly refractile lipoid deposited in the one as in the other. It is probable, too, that the more extensive repeated necrosis of glomerular tissue in nephritis is the added factor responsible for the formation of clots containing fibrin and for the excessive proliferation of epithelium, which, above all else, separate nephritis from nephrosis histologically.

The essential similarity in the nature of the two conditions is further illustrated by the experimental reproduction of each by means of the same bacterial toxin used in different quantities. Here again it is found that at least one factor concerned in the explanation of the formation of fibrin clots in nephritis is the damage produced, the larger, repeated doses of toxin causing the formation and organization of clots, and excessive regeneration of glomerular epithelium.

In the human being, the difference in aetiology between nephrosis and glomerulonephritis is probably an important factor in explaining the absence of destructive glomerular lesions in nephrosis. It is well known that infection with Streptococcus haemolyticus of whatever location, extent, or duration, is more likely to cause some degree of

nephritis than a corresponding pneumococcal infection. For example, in adults, severe acute infections which progress rapidly, such as lobar pneumonia, peritonitis, endometritis, and meningitis, whether caused by Pneumococci or Streptococcus haemolyticus, are not usually accompanied by either the clinical or anatomical manifestations of severe diffuse acute nephritis. Under these conditions outspoken nephritis is more likely to occur, however, when the infection is caused by Streptococcus haemolyticus. In the presence of more extensive infections than these, if they are of long duration, if they occur in children, and if combinations are present, such as empyema and pericarditis, organizing pneumonia, empyema and peritonitis, it is to be expected that some degree of nephritis manifests itself clinically, especially if these infections are caused by streptococci. So too, however, when such diseases are caused by Pneumococci in children, diffuse nephritis is sometimes found anatomically (18). In contrast to these intense infections, focal infections with Streptococci often cause glomerulonephritis, with its destructive glomerular lesions, while focal infections with Pneumococci appear occasionally to result in disease of the kidney, manifested by nephrosis and the absence of numerous and progressive destructive lesions of the glomeruli.

Lipoid nephrosis and glomerulonephritis, then, seem closely related in pathological physiology and anatomy. The distinctions between them in certain clinical features rest on simple and clear-cut histological differences which are probably expressions of differences in aetiology.

(*b*) *Problems in the Pathogenesis of Nephrosis and Nephritis Caused by Bacterial Toxins*

There are many questions which arise in the attempt to explain the pathogenesis of these diseases. Why, for example, are particular focal infections especially effective in the causation of nephritis, in contrast to the more violent acute infections? It may be recalled, for example, that the outspoken clinical examples of acute streptococcal nephritis, and the cases of progressive subacute and chronic glomerulonephritis, usually found in association with foci of infection in the throat or cranial sinuses, may be discovered clinically only after careful search and by the aid of bacterial cultures. The infections may indeed begin

with definite signs and symptoms of acute tonsillitis or sinusitis, which often subside, however, leaving smouldering infections followed by nephritis of increasing severity, and marked by progressive destruction of glomeruli. Even acute nephritis with scarlet fever usually develops after, not during the height of the scarlet fever itself, which suggests that a persistent, relatively asymptomatic focal infection in the throat is responsible for the nephritis. In explanation, it may be significant that in the case of chronic focal lesions the bacteria persist in the tissues unaccompanied by extensive inflammatory exudate. Toxins formed under these circumstances may be readily absorbed and carried to the kidneys. It is possible that toxins which may be formed by bacteria growing in the midst of the abundant exudate of acute infections exert their action on the inflammatory exudate, or they may be neutralized in some other manner by the cells and fluid of the exudate.

In explanation of the problem it may be of importance too that, as far as the Pneumococcus at least is concerned, the most favorable conditions for the rapid and abundant *growth* of the organisms *in vitro* are different from the conditions under which toxin formation occurs. In broth the Pneumococcus grows rapidly and abundantly when the medium is alkaline, at a pH of 7.8. As the medium turns acid, growth ceases and the formation of toxin (autolysis) begins. It is important to know, therefore, what conditions in the tissues favor rapid growth on the one hand, and formation of toxin on the other.

The nature of the toxins formed by Streptococcus haemolyticus associated with the pathogenesis of glomerulonephritis is not as yet clearly understood. The experiments of Lukens and Longcope (19) in which "glomerulitis" was produced by the injection of Streptococci into the renal arteries of sensitized animals suggest the importance of allergy in the development of streptococcal nephritis. The experimental production of progressive nephritis by means of a toxin obtained *in vitro* from these organisms has met with little success, however, and it is still necessary to study the effect on the kidney of experimental chronic infection with Streptococci and Pneumococci.

All of these problems are complex, and seem difficult to explain. It is even more obscure, however, why chronic focal infections are not accompanied by the formation of antitoxin or other immune substances sufficient to prevent the development of nephritis, whether caused by

Pneumococci or Streptococci. It may be suggested also that mixed bacterial infection plays a part in the pathogenesis of some examples of nephritis, since a variety of pyogenic bacteria is often found at the site of the chronic foci of infection which appear to be responsible for continued damage to the kidney, whether manifested by the clinical and anatomical characteristics of nephrosis or of glomerulonephritis.

Since the progressive destruction of glomeruli in nephritis depends in large part on the formation of clots within the capsules, and since the absence of this phenomenon is one of the chief distinctions between nephrosis and nephritis, it is of great interest to determine why these clots form. This problem is at present being investigated. It is pretty clear that the clots which form usually consist chiefly of fibrin. Their formation may therefore depend on the quantity of fibrinogen excreted, or may be related to the concentration in which fibrinogen escapes in relation to albumin. The clotting of fibrin, however, may largely depend on the quantity of thrombokinase liberated from repeated necrosis of tissue, necessary under any circumstances for the precipitation of fibrin from fibrinogen. It is unlikely that albumin coagulates and remains within the glomerular capsules in glomerulonephritis, and certainly this is not the case in nephrosis. Some globulins, however, consisting of molecules smaller than those of fibrinogen, but larger than the albumin molecules, may conceivably coagulate and undergo organization. This would account for the presence of coagula which are often found within the capsular spaces in many cases of nephritis, for it is often impossible to demonstrate strands of fibrin where solid coagula of foreign material are found among the glomerular tufts and within the capsular spaces.

(*c*) *Conclusions*

1. Some of the properties and the effects of pneumococcal toxin on the tissues of some laboratory animals are described. The toxin is a general capillary poison and haemolytic agent.

2. The effect of toxin in causing purpura, albuminuria, anaemia, and lesions in the spleen, liver, muscles, and kidney, varies in different animal species.

3. Intravenous injections cause leukocytosis and fever in rabbits, often followed by subnormal temperature.

4. The toxin is antigenic. Repeated intravenous injections into rabbits are followed by the formation of potent antitoxins which may be passively transferred to protect normal rabbits against both toxin and pneumococcal infection. The immunity actively acquired is relatively short in duration.

5. Forms of nephritis may be reproduced experimentally by means of the toxin corresponding, clinically and histologically, to tubular nephritis with necrosis and calcification of epithelium, acute and progressive glomerulonephritis, and to nephrosis. The quantity of toxin given, and the degree of immunity which develops during the progress of the disease are important factors in determining the character of the histological lesions in the kidney and the clinical manifestations of these forms of nephritis.

6. The chief anatomical distinction between nephrosis and glomerulonephritis, whether in man or in experimental animals, is the absence of numerous obstructive glomerular lesions in nephrosis. In glomerulonephritis the glomeruli are progressively obliterated by the organization of exudate and excessive regeneration of epithelium.

7. In progressive glomerulonephritis, produced by many repeated injections of toxin, albuminuria, cholesterolaemia and necrosis of tissue diminish, while increasing nitrogen retention appears. There is evidence to indicate that the increasing nitrogen retention of progressive nephritis, the result of obliteration of glomeruli by healing processes (scarring), occurs at a time when there is a relatively high degree of immunity to the effects of toxin in producing fresh necrosis of tissue, massive albuminuria, and cholesterolaemia.

8. The functional disorders (anuria, oliguria, albuminuria, and haematuria) cannot be definitely correlated with histological changes in the kidney, either in human or experimental nephritis.

9. There is experimental evidence to suggest that increased production of cholesterol is concerned in the mechanism responsible for the cholesterolaemia of experimental pneumococcal nephrosis and nephritis. The highest degree of cholesterolaemia is produced in normal animals without any immunity. As immunity is developed by repeated injections of toxin, the cholesterolaemia decreases parallel with the disappearance of other known effects of the toxin, *i.e.*, necrosis of tissue and albuminuria.

10. Experiments are described showing the importance of widespread capillary damage and loss of plasma protein into the tissue spaces and body cavities in the pathogenesis of oedema in nephrosis and nephritis.

11. It is shown that oedema (the accumulation of plasma protein together with fluid in the tissues) is one of the mechanisms responsible for the reduction of the total plasma proteins.

REFERENCES

(1) Blackman: *Bull. Johns Hopkins Hospital*, 1934, **55**, 1 (July).
(2) Cole: *J. Exp. Med.*, 1914, **20**, 346.
(3) Avery and Cullen: *Ibid.*, 1920, **32**, 547, 571, 583.
(4) Julianelle and Reimann: *Ibid.*, 1926, **43**, 87.
(5) Parker: *Ibid.*, 1928, **47**, 531.
(6) Schade und Claussen: *Ztschr. klin. Med.*, 1924, **100**, 363.
(7) Iversen: *Verhandl. deut. Gesells. Kreislaufforschung*, 1929, **2**, 133.
(8) Verney: *J. Physiol.*, 1926, **61**, 319.
(9) Fishberg: *J. Biol. Chem.*, 1929, **81**, 205.
(10) Hiller, Linder, Lundsgard and Van Slyke: *J. Exp. Med.*, 1924, **39**, 931.
(11) Peters and Van Slyke: *Quantitative Clinical Chemistry*, 1932, **1**, 218.
(12) Parker: *J. Exp. Med.*, 1929, **50**, 161.
(13) Blackman, Brown and Rake: *Bull. Johns Hopkins Hosp.*, 1931, **48**, 74.
(14) MacCallum: A Text-book of Pathology, 1932, 139.
(15) Osler and McCrae: The Principles and Practice of Medicine, 1924, 91.
(16) Forbus: *Arch. Path.*, 1926, **2**, 318
(17) Rich: *Bull. Johns Hopkins Hosp.*, 1930, **47**, 365.
(18) Blackman and Rake: *Ibid.*, 1932, **51**, 217.
(19) Lukens and Longcope: *J. Exp. Med.*, 1931, **53**, 511.

EXPLANATION OF ILLUSTRATIONS

(Photographs by Mr. Milton Kougi)

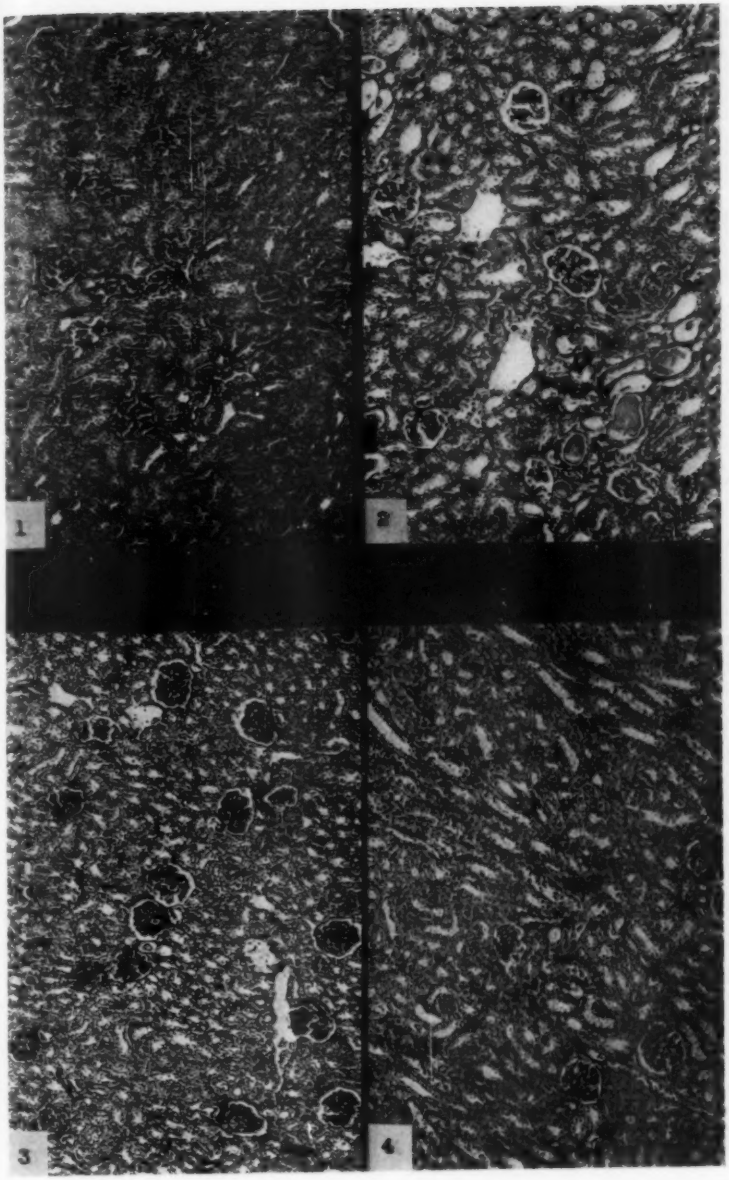
FIG. 1. Photomicrograph of a section of the kidney of a normal rabbit, R-26 (expt. 34).

FIGS. 2, 3, AND 4. Experimental nephrosis. These photographs show changes in the epithelial cells, and in the calibre of the tubules. The glomerular capillaries look normal. There are no clots of exudate. FIG. 2. Kidney of rabbit 7 (expt. 34). FIG. 3. Kidney of rabbit 23 (expt. 34). FIG. 4. Kidney of rabbit 14 (expt. 34).

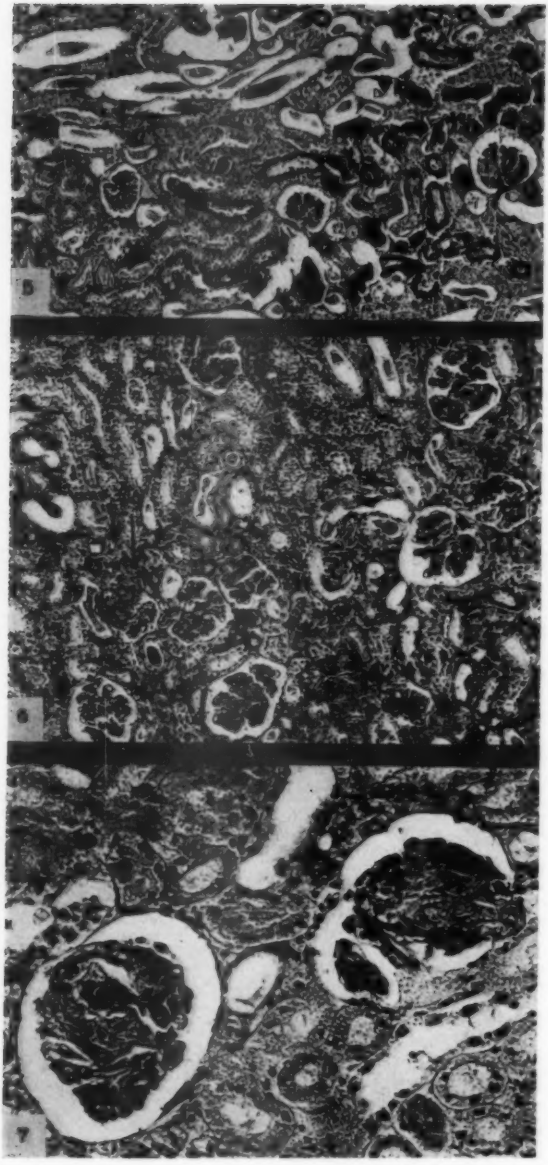
FIG. 5. Photomicrograph of kidney of rabbit 12 (expt. 2), three days after a single intravenous injection of 1.1 cc. of pneumococcal toxin (1 M.L.D.). There are no visible lesions in the glomerular capillaries, and no clots of exudate are present. Many of the tubules are lined with thin, regenerated epithelium. There is widespread calcification of necrotic tubular epithelium. The section is stained with haematoxylin and eosin. The calcium looks black in the photograph.

FIG. 6 (Expt. 34, R-15). Progressive glomerulonephritis. Kidney at the end of 15 days. The glomeruli are large, compact and cellular. The magnification is the same as that of the tissues shown in figs. 1, 2, 3, 4 and 5. The arrow points to an epithelial crescent. The cellular appearance of the glomeruli depends chiefly on changes in the epithelial cells over the capillaries. Some capillaries are occluded by thrombi. Between most of the tufts there are collections of cells and exudate.

FIG. 7 (Expt. 34, R-52). Progressive glomerulonephritis. Kidney at the end of 9 days. High magnification showing partly organized masses of hyaline exudate between tufts of glomerular capillaries.



FIGS. 1–4



FIGS. 5–7

INSOMNIA IN CLINIC PSYCHIATRIC PRACTICE

WENDELL MUNCIE, M.D.

From the Henry Phipps Psychiatric Clinic, The Johns Hopkins Hospital

Submitted for publication, January 29, 1934

HISTORICAL CONSIDERATIONS AND AIMS OF THIS COMMUNICATION

Sleep has been the object of so much fruitful investigation from the standpoint of its physiological and pathological aspects and its therapy that some good reason should be advanced for a general discussion of its occurrence in pathological mental states. Our interest in the subject arose at first from the necessity for meeting the issue it constantly presents in the practical handling of patients on the wards of the Henry Phipps Psychiatric Clinic, The Johns Hopkins Hospital. In contrast to insomnia seen on medical and surgical services, usually an incidental matter and amenable to rather casual therapeusis, insomnia in mental disease offers peculiar and special difficulties. The coöperation of many patients can not be taken for granted as is usually possible in general hospital services. The relative chronicity of mental illnesses offers further obstacles. The group life which is the cornerstone of most modern clinic treatment demands that the nights be as undisturbed as possible.

Our further interest was in an attempt to assimilate insomnia, as an item of psychiatric interest, into the body of general psychopathology.

An excellent exposition of the physiology, psychology, pathology and therapy of sleep with a review of the literature to date is to be found in the report of the Thirty-first German Congress for Internal Medicine (1) held in 1914 at Wiesbaden. The approach is rather formal and nosological and an idea of the vagueness of the status of insomnia as an item of general psychopathology may be gained from Goldscheider's statement that among other conditions hypnotics may be given "in gross excitements and in psychoses," without further differentiation.

In 1924 again the subject was treated exhaustively in a somewhat

similar manner by Lhermitte and Tournay (2) at the International Neurological Reunion in Paris. At that time narcolepsy received a great deal of attention. In 1931 Lhermitte (3) published his monograph which deals only in passing with the problem as we meet it. It is an excellent source book for the current theories concerning the mechanism of sleep, the structures involved, and especially rich in clinical material drawn from the encephalitis epidemic and from that group of cases first described by Lhermitte as "hallucinose pédonculaire" purporting to point to the structurally localizable nature of sleep and dreams.

"Der Schlaf" (1929) (4) under the general editorship of Sarason seems to be the best of the more recent monographs on the subject. Here we see again the same all-round attack on the problem. That the psychopathology of the subject has made little progress is seen in the reiteration of Goldscheider's guiding principles, as quoted above.

The nearest approach to what we feel are the needs of the psychiatrist in dealing with this problem is to be found in the excellent article by Laudenheimer (1930) (5), the clinic by Pollock (1930) (6), the monograph by Gillespie (1929) (7), and the earlier publication by Ladame (1923) (8). Further reference to these publications will be made.

In the present brief communication we will attempt to trace the general psychopathological principles operating in insomnia, and add some practical hints as to treatment, based on observations made on the wards of the Henry Phipps Psychiatric Clinic.

METHODS OF STUDYING SLEEP

Sleep has been studied among other ways in respect to (1) its structure, (2) its depth, (3) the physiological chemical changes occurring in, and the influence of drugs on it, and (4) its content (dreams). By its structure we may include what may be observed as regards onset, duration, and ending. Ladame (8) kept accurate records on patients in a psychiatric hospital, dividing the night into (1) "temps latent"—the time elapsing between going to bed and going to sleep; (2) "temps intermédiaire"—the short periods of waking throughout the night; (3) "temps de sommeil effectif"—the total duration of sleep in a night; (4) "temps matinal"—the time elapsing between waking

and arising. Ladame showed the individuality of the sleep curve, the frequency of minor irregularities, and stated that insomnia attacked first the "temps latent" and the "temps matinal," and that the "temps intermédiaire" was least affected. His is a painstaking record and would involve more detailed reporting than is possible as a routine measure. Lévy-Valensi (9) uses a clinical classification of sleep disturbances much like Ladame's.

Guttman (10) used the "aktogramme" as a means of giving information concerning sleep by inference from the gross bodily activity measured by a tambour on a drum tracing. It is not easy to interpret such a record, as it carries inferences concerning both structure and depth. Furthermore, it is hardly applicable to the observation of all the patients in a clinic at any time.

Richter (11) has studied the depth of sleep by the skin resistance method, which would seem to have the inherent virtue of being the least bothersome to the sleeper. He reviewed the earlier work of Kohlschütter (12), Michelson (13), and others concerning the establishment of types of sleep—early deep sleep (later called type "A"), and later deep sleep (called type "B"), based on the degree of auditory stimulus necessary to waken the sleeper. He also reviewed the findings of MacWilliam (14) on the blood pressure and restlessness which divided sleep into that with a lowering of blood pressure (good sleep with relaxation), and sleep with a heightening of blood pressure (poor, light, restless sleep with tension). Richter found that the individual variations in the type of sleep as judged by its depth were much greater than had been supposed, although there were types approximating the foregoing. He also found a striking parallelism with the suggestions contained in MacWilliam's work, namely, in relaxed sleep the palm-palm resistance was very high; in tense sleep it remained low much as in the waking state.

We owe more recent data of the physiological chemistry and pharmacology of sleep to Cloetta, Demole, Pick, Hess, Wuth, and others. This has been summarized by Sarason (4), Lhermitte (3), Gillespie (7) and recently by Katzenelbogen (15). The review of hypnotics by Wuth (16) can be recommended for its practical hints.

The dream material has not entered into this investigation and we therefore only in passing mention the divergent views of the psycho-

analytic school and of Lhermitte's experiences, especially with "hallucinose pédonculaire" as summarized in his monograph. Lhermitte, in effect, looks upon dreams as the positive aspect of sleep, and as due to the positive activity of the peduncular region at the level of the nucleus of the third cranial nerve.

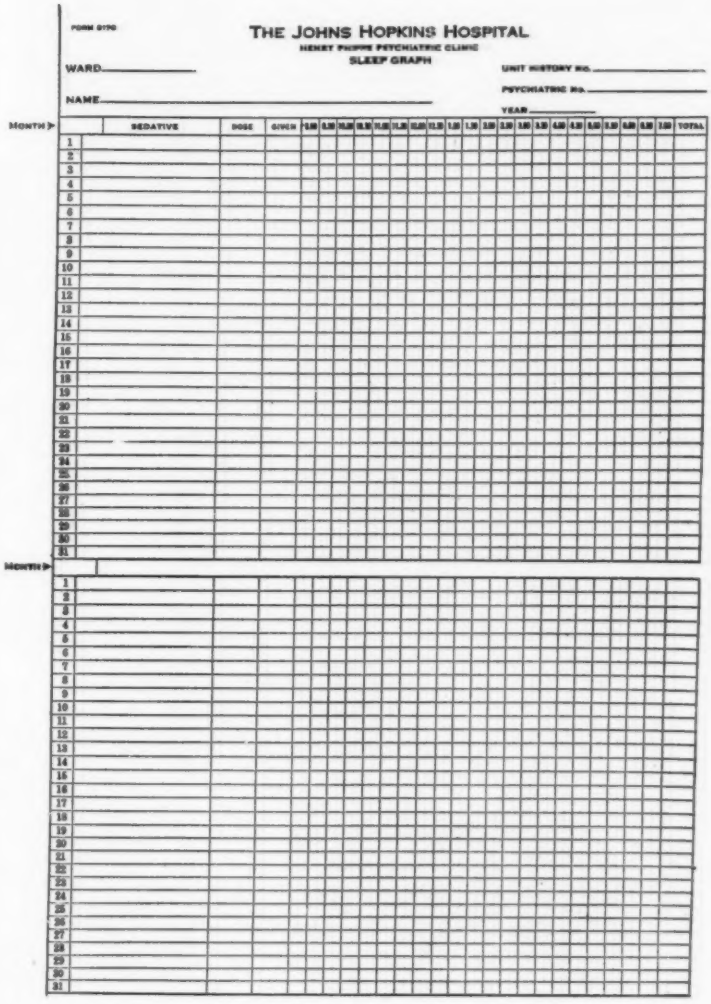
METHOD OF THE PRESENT INVESTIGATION

It has always been the practice at the Henry Phipps Psychiatric Clinic to note at half-hourly intervals throughout the night whether the patients are awake or asleep. These nurses' records are available to the physician each morning, and the further verbal amplifications give descriptive hints as to the character of the sleep. The curve of total hours of nocturnal sleep is recorded as a continuous line near the bottom of the behavior chart. It occurred to the superintendent of nurses at this clinic, Miss Bernadette A. Mullin, and to the writer, that perhaps more could be learned as to quality and quantity if the information of each night were charted on a permanent record for each separate patient. Accordingly, the accompanying chart (fig. 1) was devised and has been in use since July 1, 1930. This chart has several advantages:

1. It gives immediate information concerning the time and duration of sleep.
2. It gives an immediate correlation between sleep and hypnotic medication.
3. It leaves room for marginal annotations, and cross references to other parts of the history, *e.g.*, the behavior chart, the pulse and the temperature chart.
4. It is concise, one sheet of the size of ordinary history paper giving space for two months, and adds little bulk to the history—a matter of no little importance.
5. Its use involved no change in nursing procedure.
6. The recording is easy and takes little of the nurse's time.

It has several disadvantages:

1. It is based merely on our half-hourly type of recording.
2. It gives no information concerning the depth of sleep.
3. It gives no information concerning the condition and activity when the patient is not asleep.


FORM 0170

THE JOHNS HOPKINS HOSPITAL

HENRY PHIPPS PSYCHIATRIC CLINIC

SLEEP GRAPH

WARD_____ UNIT HISTORY NO. _____

NAME_____ PSYCHIATRIC NO. _____

YEAR _____

MONTH ▶ | SEDATIVE | DOSE | GIVEN | 7.00 | 7.30 | 8.00 | 8.30 | 9.00 | 9.30 | 10.00 | 10.30 | 11.00 | 11.30 | 12.00 | 12.30 | 1.00 | 1.30 | 2.00 | 2.30 | 3.00 | 3.30 | 4.00 | 4.30 | 5.00 | 5.30 | 6.00 | 6.30 | 7.00 | TOTAL

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

MONTH ▶

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

FIG. 1

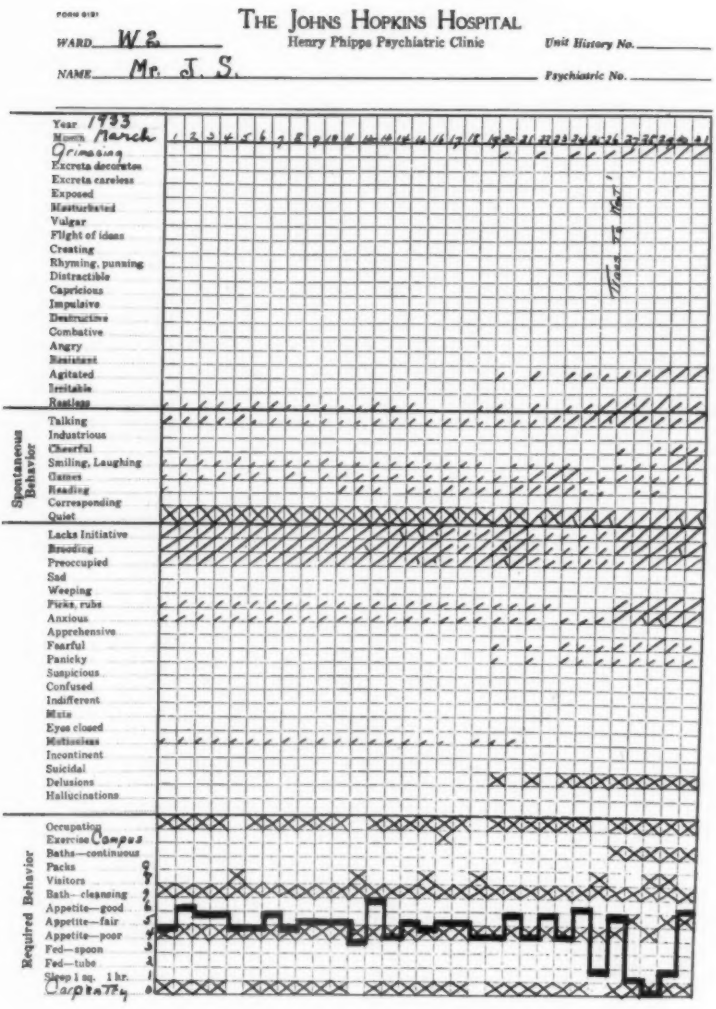


FIG. 2. THE SLEEP GRAPH

The nurses' notes kept nightly on each patient have to supplement the record.

In general, relaxed posture, deep, regular respiration (and snoring), unresponsiveness to environmental stimuli have to serve as the criteria for sleep. Fairly uniform dependability is attained.

Figure 2 shows the general behavior of the patient, J. S., for a month, and figure 3 his sleep for the same period.

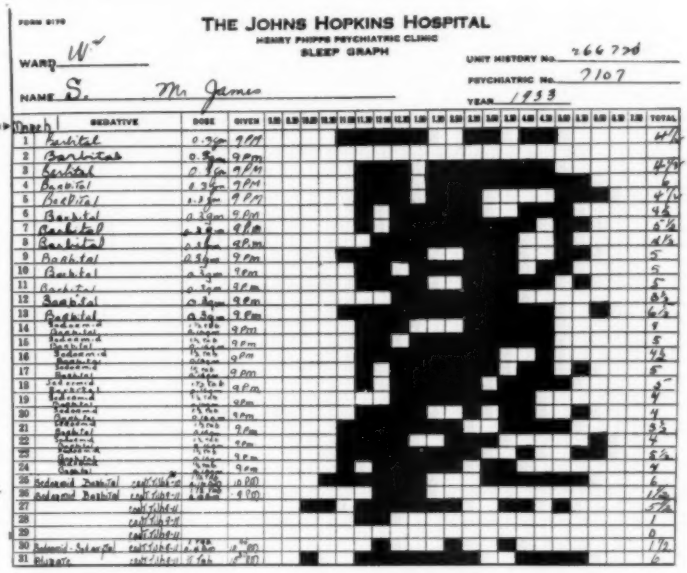


FIG. 3

The duration and continuity of sleep and waking are read first. In addition, from each patient who presents insomnia as a subjective or objective complaint, we try to get an historical picture of his normal sleep habits including the foregoing features, as well as the depth of sleep, dreams, satisfaction from sleep, and the modifying factors. In such a way we attempt to make a comparison between the observed and subjectively evaluated clinical data and the usual sleep curve.

TYPES OF SLEEP AS DETERMINED BY THIS METHOD

These charts have corroborated Ladame's findings of a certain individuality of the sleep curve and the presence of transient minor irregularities from environmental factors.

In addition, the charts have shown the following persistent disturbances in sleep: (1) difficulty in falling asleep, (2) early waking, (3) intermittent sleep, and (4) total sleeplessness. These correspond to Levy-Valensi's groupings and to Ladame's findings.

GENERAL PSYCHOPATHOLOGICAL CONSIDERATIONS

For a correlation with a scheme of general psychopathology we consider the following general considerations used in Adolf Meyer's teaching (17):

"1. What mental factors and imbalance may do along lines of
   (a) *Minor interferences or complaints* (wrongly called "neuroses") such as special preoccupations and substitutions and dissociations with attention to the behavior and rôle of special experiences and their hangovers or anticipations.
   (b) *Dominating affects* and their fixation (pure or impure) with *incidental modification of content.*
   (c) *Leading topical content disorders* from projection, disowning tendencies, compensation, systematization, and false crystallizations and fixations.
   (d) *Specific disorders of grasp*, and panic, and puzzle states.
   (e) *Developmental deficiencies.*
2. What *poisons and metabolism and special organs and their malfunction can do to the brain and its functions and thereby to the personality* (delirious and delirioid delusional mal-support reactions).
3. What *leading structural disorders* of the brain can do to the personality:
   (a) as general developmental deficiencies;
   (b) towards disorganization of the psychobiological functioning."

In accordance with these principles, the sleep disturbances are considered as due to, or associated with:

1. *Minor incidental or inherent personality problems*, with hypochondriacal, hysterical or other merergastic expression.

2. *Affect fixation*—in their relatively pure states of diffuse sadness and melancholy, and of elation.

3. *Content* (topical) disorders
   (a) Involving the impure affects of tension, anxiety, fear, suspicion, etc.
   (b) The more specific content disorders (without affect), in paranoid and schizophrenic development with projections (delusional or hallucinatory), crystallizations and systematizations.

4. *Poor support of the brain* functioning through toxic, infectious, metabolic disturbances (as in delirium).

5. *Structural, organic damage*, as in senile processes, arteriosclerosis, paresis, *etc.*

THE RESULTS OF THE INVESTIGATION

These general psychopathological principles as applied to the sleep records of our patients have shown:

(1) In the merergastic or "complaint" disorders, we commonly find difficulty in falling asleep, and broken intermittent sleep, with or without reduction in the total number of hours. In this group we deal with content material, in which disturbance in sleep is incidental to anxiety, fear, obsessions, or restlessness or a substitutive phenomenon of an hysterical or hypochondriacal nature. In this group we deal with the "sleep pedants" and "sleep hypochondriacs" of Laudenheimer—those panicky about sleep, patients with fixed ideas concerning the need for sleep of a certain quality and quantity, with fears of insanity when the sleep is unsatisfactory for any reason, with comparisons with the sleep of others, *etc.* Especially interesting is that group for whom insomnia becomes the sufficient excuse for any failures and deficiencies in the life adjustment (see fig. 4).

(2) Fixations of mood of,

(a) The depressive type (content subordinate) produces characteristically the disturbance of early morning waking, with difficulty in returning to sleep. There is little or no difficulty in falling asleep, and the sleep is fairly continuous up to the period of waking, which may be from one to several hours ahead of the usual waking time. This type of disturbance is best seen in the simple retarded depressions with the diffuse affect of sadness, blueness, melancholy, best typified in the "rural" depressions. This type of disturbance may be regarded

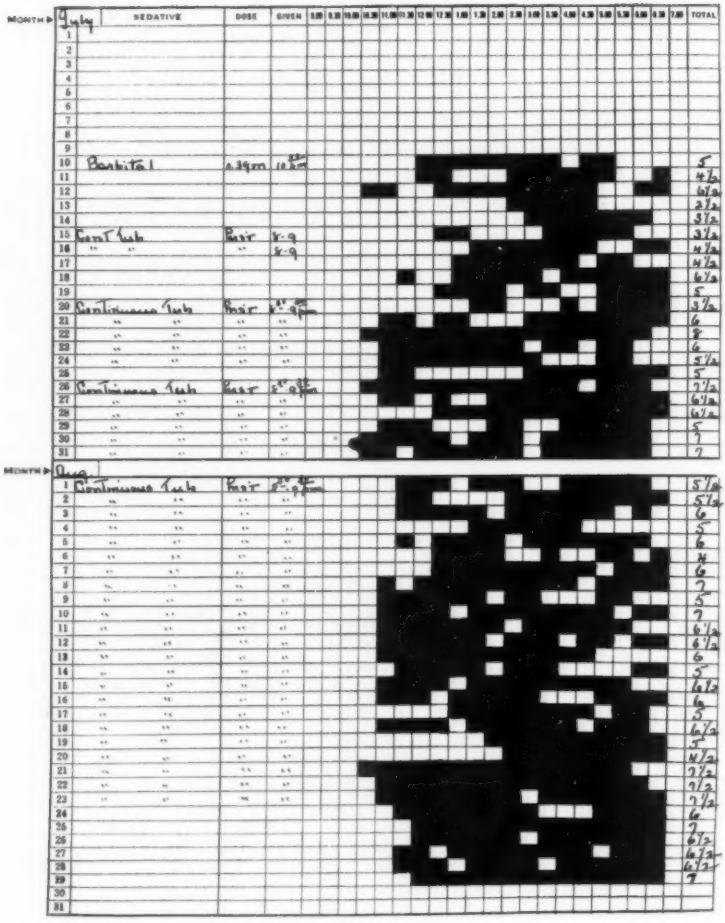


FIG. 4. WOMAN, AGED 46, WITH COMPLAINT OF INSOMNIA AND FATIGUE—AN HYPOCHONDRIACAL REACTION

Barbital was given on the first night through an error

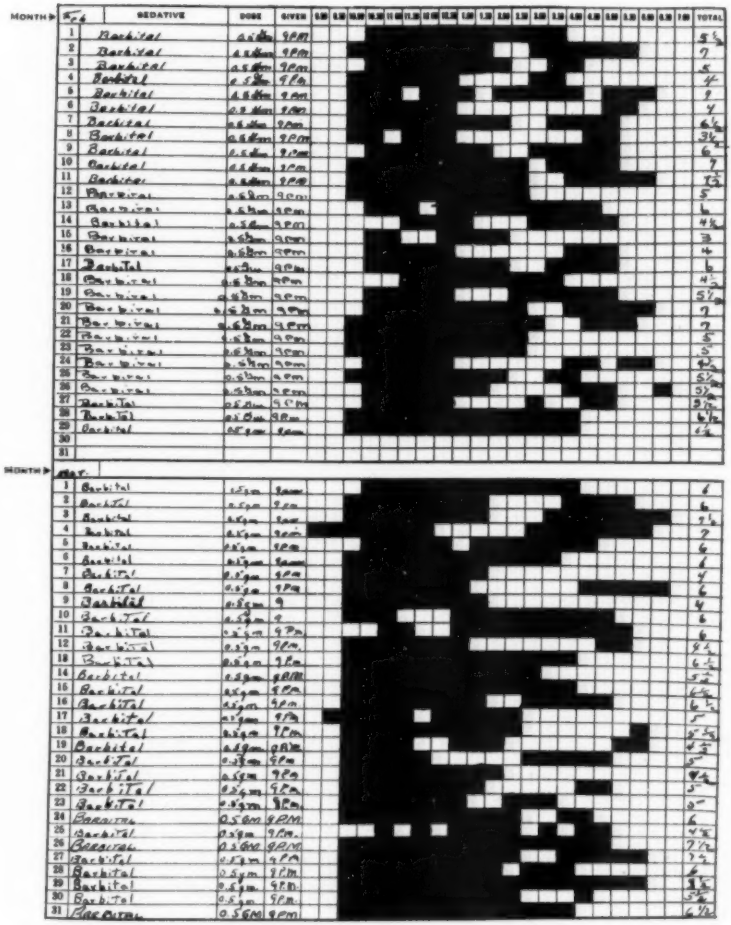


FIG. 5. WOMAN, AGED 60 YEARS, IN ELEVENTH ATTACK OF DEPRESSION, WITH MARKED SLOWING, INDECISION, DEPRESSED MOOD, AND TYPICAL MORNING-EVENING VARIATION OF MOOD

Early waking the rule. The patient complained of indecision and sleeplessness. "Depression" was expressed largely in the general activity, to a lesser degree as a complaint of mood.

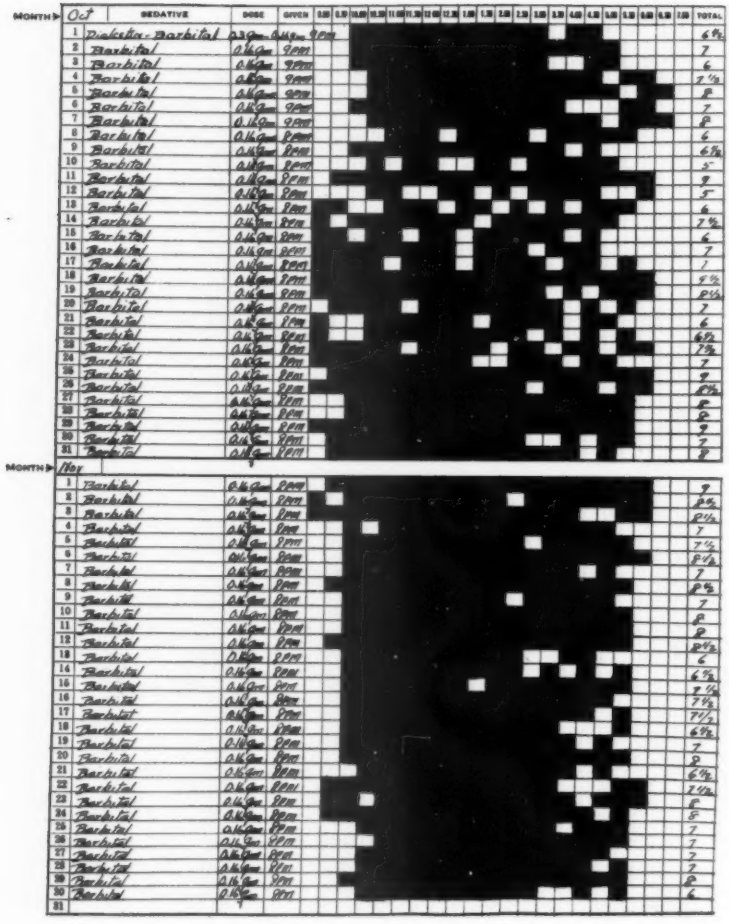


FIG. 6. MAN, AGED 53 YEARS, in SECOND ATTACK OF SIMPLE DEPRESSION

Early waking the rule. Note the episode of about 14 days duration in October when broken sleep is added to early waking. This coincided with coming to fore of strong topical material of a sex type, with demands for freedom to go to prostitutes, etc.

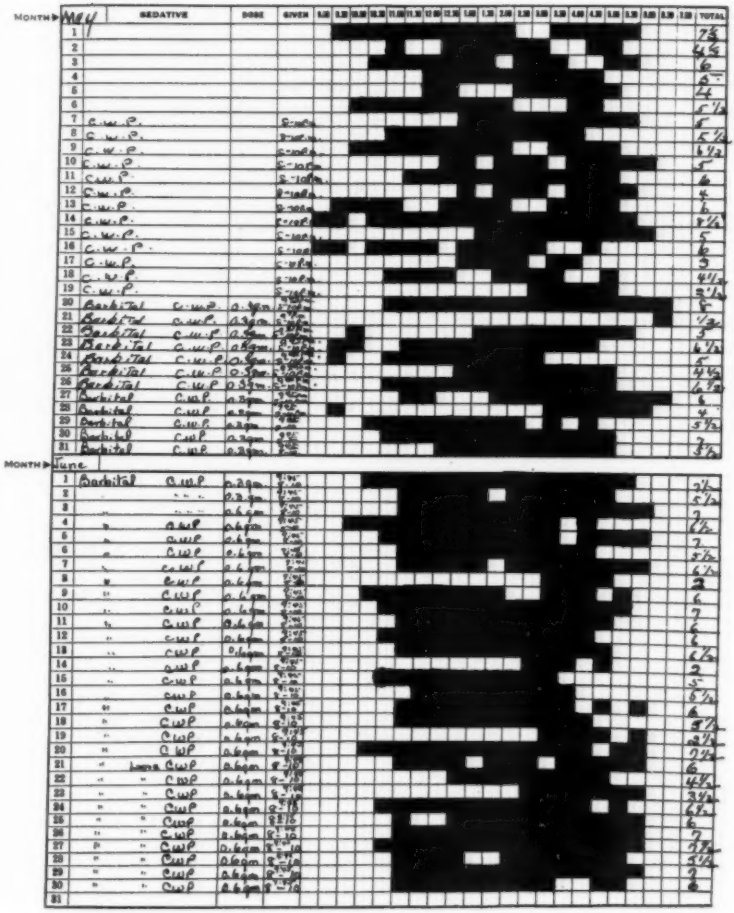


FIG. 7. MAN, AGED TWENTY-SEVEN YEARS, SCHIZOPHRENIC, WITH CONTINUOUS AUDITORY HALLUCINATIONS OF A PLEASANT CHARACTER AND EROTIC CONTENT. MOOD NEUTRAL OR EUPHORIC

Difficulty in going to sleep, and almost sleepless nights

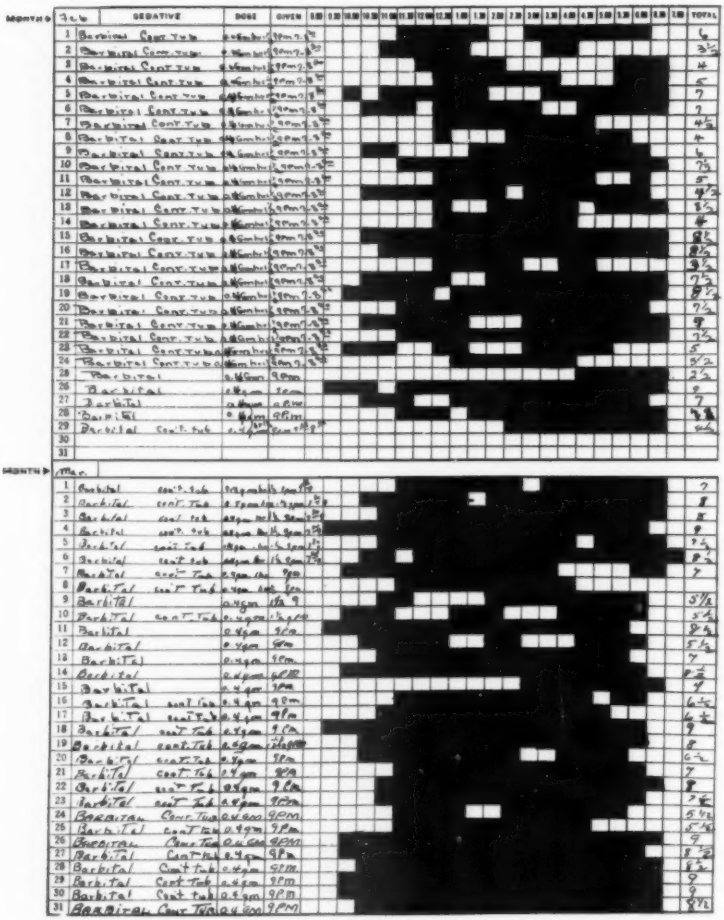


FIG. 8. WOMAN, AGED TWENTY-FOUR YEARS, PANICKY, WITH MANY IDEAS OF REFERENCE, AUDITORY MISINTERPRETATIONS, AND PASSIVITY FEELINGS. DEPRESSED MOOD

Second hospitalization within 3 years but recovery was incomplete after the first hospitalization. Difficulty in falling asleep and restless intermittent sleep.

as one of the pathognomonic signs in depressions. Pollock (6) also stresses this point, which we believe deserves more consideration as a diagnostic sign, but also as another means of approach to the profound change in body economy produced in depression (see figures 5 and 6).

(b) The elated type gives no certain picture, all varieties of disturbance being noted in the same patient without any consistency.

(3) Content disorder (topical rather than diffusely affective), from whatever cause, introduces difficulty in falling asleep and intermittent sleep (and occasionally "white nights") as the characteristic disorders.

This is also seen in a transitional form in depressions characterized by *worry and anxiety* as opposed to diffuse sadness. The patient tells us that at the beginning of his trouble he worried and could not fall asleep with his accustomed ease. Later as the depression deepened he found that he fell asleep more easily but then wakened unusually early. In schizophrenic hallucinosis and paranoid states (without affect) falling asleep is apt to be retarded because of the inability or unwillingness to relax sufficiently to fall asleep. In schizophrenic hallucinosis it is not rare to have the patient intimate a preference for staying awake and listening to voices instead of falling asleep; or one may deal with a dread of sleep because of ideas of influence, nightmares, fear of emissions, of not waking again, *etc.* When good sleep is present associated with serious content disorder, one may suspect the deliberate utilization of sleep to obliterate the content, or an apathetic, unconcerned attitude toward the content. Pollock agrees in principle with this view (see figures 7 and 8).

(4) In support disorder from toxic-infectious-metabolic sources, sleep may be increased as a direct result of the action of the toxin and may then be reduced in an intermittent fashion or even entirely with a further increase in the toxin or with the addition of new elements producing various forms of imbalance: from exciting fear and hallucinosis to muttering, twitchings and vestibular disorders, and then may go over into coma in terminal states. The true picture of the sleep in support disorder demands that a twenty-four hour record be kept (which anyhow should be provided, for day sleep also counts). In support disorder fearful content is frequently present, and in these

cases the sleep difficulties noted in (3) above are also seen. This is a complicating factor in treatment as will be noted later (see figure 9).

(5) In organic states, notably senility and arteriosclerosis, according to Pötzl (18) and in accord with our general experience, there is often catnapping by day and greatly reduced sleep by night, with waking occurring only shortly after going to sleep, giving a "caricature" of the "A" type sleep (see page 138). Our case material is not large enough to allow of definite conclusions. It is perhaps worth mentioning that several patients suffering from classical involutional melancholia

FIG. 9 WOMAN, AGED 75 YEARS IN HYOSCINE AND BROMIDE DELIRIUM

Day and night sleep is charted showing the intermittent character of the sleep, and the gradual approach to normal sleep habits with the progress of treatment (reduction of blood bromide by forcing fluids and sodium chloride).

in whom organic deficit appeared did not present the type of sleep disorder as seen in simple depressions, but rather broken, intermittent sleep,—and great difficulty in going to bed and getting to sleep. Moreover, nocturnal perplexity and epileptoid attacks of violence are complicating features.

These results may be compared with those of Pollock (6), which may be summarized as follows: (1) Early waking is pathognomonic of depression; (2) difficulty in falling asleep is seen most often in the "neuroses" (for example in anxiety states), and is coupled with the ability to sleep late in the morning; (3) ability to sleep well in the

presence of worries and other topical concerns is an ominous sign of inadequate emotional response of the organism; (4) sleep is incompatible with active delirium.

TREATMENT OF INSOMNIA

In any illness there is a great urge to give whatever symptomatic comfort is possible, and this applies equally well to mental disease. We give diets and cathartics for constipation, analgesics for headaches, hydrochloric acid or bicarbonate of soda for stomach discomforts. Why not then do something for insomnia? Something is done, of course; yet we find a great deal of discord prevailing as to the proper symptomatic treatment. Over the question of hypnotic drugs the issue is joined between therapeutic nihilism and over-enthusiasm, fear of habituation and the objection to a mere covering up of the fundamental difficulty. The treatment of insomnia must develop from a consideration of the origin of the difficulty in exogenic, organogenic, neurogenic, psychogenic and constitutional factors. A practical aid is also at hand in the determination of the degree of the basic involvement as a minor or major psychosis, and in the careful estimation of the constitutional factors for stability, dependability, self-reliance and frankness.

In general we agree with Straszer (19) on the preference for hydrotherapy over drugs, but also with the same author on the usefulness of the two in combination, and always with psychotherapeutic effort. One cannot simply order tubs, or packs, or drugs—there must always be a psychotherapeutic effort in the establishment of good working rapport and mutual understanding and optimistic suggestion.

From what follows it will be seen that the administration of drugs in psychoses is not the uncomplicated matter Goldscheider's postulates indicated.

There are a few general rules in regard to the administration of hypnotic drugs which should be observed:

(1) The administration should always be the direct responsibility of the physician, and should not be left to the discretion of nurses or of the patients themselves. This rule is in contrast to a common custom of leaving "if necessary" orders (too often interpreted on the basis of nursing convenience), and of leaving medication at the bed-

side to be taken if not asleep by a set time. We have rarely seen any good come from the latter practice, the conflicts engendered by the necessity of making the choice outstrip any good the drug might accomplish.

(2) Medication should be given in such form that its identity and quantity does not preoccupy the patient. A practical way is to administer all hypnotic drugs in a standard amount of elixir, or in a capsule with a neutral filler. This will discourage morbid curiosity and hypochondriacal preoccupations.

(3) The danger of addiction even to "harmless" drugs must always be kept in mind, the addiction having little or no physiological component in most cases. The danger of addiction is greatest in patients with a past history of chronic instability and an attitude which seeks external help for internal troubles. It is wise to come to an early estimate of the dependability of the patient and to refuse palliative medication to the undependable, reserving drugs for emergency treatment and as a "lift."

(4) Reduction of the drug should be attempted at frequent intervals. Psychotic symptoms may reappear with sleep disturbance, after being previously masked by the influence of the drug. Reduction should be gradual, in successive steps a week apart, the administration of the elixir or capsule being continued for a few days in order to avoid fear of the withdrawal and to determine the actual need for the hypnotic action of the drug. If sleep disturbance reappears, when sleep was previously good, the patient may be able to accept the factor of suggestion.

(5) Know the toxic symptoms and constantly be on the lookout for them. If an ordinary dose of medication does not produce the desired effect, even the reverse, do not use that drug again. Diurnal drowsiness and dulling, dizziness, and incoördination, diminished throat and corneal reflexes should always make one suspect the medication.

(6) Never allow a patient who is still being given drugs to leave the hospital. It is time well spent for him to remain in hospital until all drugs are unnecessary and he is aware of and can accept that fact.

Briefly, we have found the following procedures useful in the treatment of insomnia:

(1) In the complaint disorders and minor psychoses where sleep becomes the object of concern and its disturbance a substitutive phenomenon, we deal with obsessive fears, hypochondriasis, and with hysterical reactions. Laudenheimer speaks of "sleep pedants" and "sleep hypochondriacs."

In such cases the disturbance in sleep is so closely connected with and a part of the personality disturbance with its basis in constitutional features and life experiences, that its treatment has to be an integral part of the adjustment of the personality. This comes about through some type of personality study—an analytic-synthetic approach designed to give the patient new weapons with which to adjust false perspectives and old habits, fears, anticipations, memories, *etc.*

Symptomatic treatment is mainly by explanatory reassurance and suggestion, planning of the day's activities, formulas to aid relaxation, and hypnosis. These fail unless accompanied by personality study with its more inclusive and far-reaching goals by which the immediate help becomes consolidated for future use. We avoid drugs because of the danger of undue dependency and addiction in such constitutional personality grounded illnesses. Here again we differ from much current practice (20, 21). These cases are so intensely individual that satisfactory therapeutic generalizations are less easily made than with the other groups mentioned.

(2) In states of fixation in a depressive mood with early waking, we depend on hydrotherapy (continuous tubs or cold wet packs) supplemented, when necessary, by barbital in nightly doses of from 0.15 gm. to 0.6 gm. The drug tends to prolong the sleep and to facilitate return to sleep when wakened. The results are usually gratifying both as to the improvement in the sleep and the amelioration of depressive distress.

In this connection it may not be amiss to note that all recent chemical hypnotic research has been with the goal of producing quickly-acting, easily excretable, non-toxic drugs. Hence the great number of complex and unsaturated barbiturates to replace barbital (amytal, dial, alurate, and others). In view of the great numbers of depressed patients for whom early waking is the prevailing disturbance rather than difficulty in falling asleep, there is a clear need for a non-

toxic, easily excretable drug whose action is delayed. The sulphone group is too undependable for routine use, although it tends to produce a "B" type of sleep. Barbital stands intermediate between type "A" and type "B" in its action. We have had little success with giving drugs to such patients only after waking. We therefore give an adequate dose of the medication at the usual time, one half hour before retiring.

In manic excitements we have had our best results from the diurnal use of hydrotherapy,—continuous tubs or cold wet packs instituted early in the illness and increased or decreased as necessary—coupled with sodium amytal 0.4 to 0.8 gm. (by mouth) or paraldehyde 6 cc. to 12 cc. (by mouth) at night.

There is less expectancy of the production of well regulated sleep in manic excitements than in depressions.

(3) In content disorders we differentiate between the sweeping reactions of fear, panic, catathymic, paranoid and schizophrenic states and the more minor personality-determined states with anxiety, obsessions and ruminations (see (1) above). In the former are to be found the cases of sleep disturbance most refractory to treatment. In fact, we have learned one lesson: when such a patient does not want to sleep, the only way to guarantee "sleep" is by the induction of general anesthesia or narcosis. Ordinary doses of hypnotics are customarily of no use. In one such patient actively hallucinated and overactive, completely sleepless for 5 days and 4 nights in spite of many hypnotics used singly and in combination, we broke the "vicious circle" with[1] avertin administered rectally. This gave a prompt short "sleep," and afterward the patient slept well at night without any medication whatever. It is in such cases that the question as to the advisability of sleep treatment arises. This problem would take us far afield and can not be discussed here.

In milder cases as in tension depressions, barbital administered orally in 0.1 gm. to 0.15 gm. doses two to four times during the day may reduce the tension to such a point that sleep may ensue without an hypnotic dose at night. This is often more effective in producing good sleep than giving the hypnotic in a single dose at night.

[1] Goldscheider: *loc. cit.*

(4) In support disorders, we depend largely on hydrotherapy for its calming and eliminative effect on a toxic system. We give drugs only when hydrotherapy alone fails and clinical evidence points to sleeplessness as contributory to the condition (as in delirium tremens), and when there is a reasonable expectation that general improvement would result from a temporary cessation of the restlessness and fear incidental to the toxic state.

Many support disorders in patients coming to this clinic are induced, as the result of the failure of some hypnotic drug to produce sleep (usually in the treatment of a content disorder) and the steady increase in the dose taken. Bromide delirium, seen not uncommonly in psychiatric practice, may be used as an illustration.

Observation of the twenty-four hour sleep chart, rather than exclusive focussing on the nightly chart, will reduce the number of cases of support disorder that are considered to be in need of drug treatment.

It is to be remembered that sedative and hypnotic drugs often produce a paradoxical effect in toxic states; at least the danger from increased toxicity from their use is marked in support disorders. Therefore, when a drug is used, it should be in moderate doses of the most effective, least toxic, least cumulative possible—as, for example, paraldehyde (4 cc. to 8 cc.), sodium amytal (0.2 gm. to 0.4 gm.), alurate (0.1 to 0.2 gm.), dial (0.1 to 0.15 gm.), sedormid (0.3 to 0.4 gm.). The carbamides possess a theoretical advantage over the barbiturates in their more complete oxidation in the body, with a consequent decrease in the danger of intoxication (21).

When fearful content presents an emergency, and when reassurance and hydrotherapy fail, larger doses of the same drugs may well be employed. Spinal drainage sometimes seems to reduce the excitement of delirium tremens. In support disorders, good nursing care designed to get done what is necessary without antagonizing the patient, and the reduction in the opportunity for environmental misinterpretations through simplicity of setting and attention, and relative isolation, elimination of suggestive shadows and wall patterns and reassurance to allay fear are more valuable in general than any drug therapy.

(5) Our experience with organic defect conditions has been limited. In general we have been guided by the same considerations as in (4)

above, keeping in mind the ease of intoxication by drugs in organic disease of the brain. This perhaps is linked with the increase in meningeal permeability in such cases. Continuous tubs, even cold wet packs when the cardiovascular apparatus is in good functional condition, are to be preferred to drugs. General narcosis with paraldehyde or amytal, or other barbiturate may be necessary for the excitements of paresis, epilepsy, etc.

SUMMARY

As psychiatrists, we undertake the obligation of understanding to the best of our ability the behavior of our patients. Controlled sleep records may be an important help. The simple chart illustrated here is capable of yielding considerable information of diagnostic importance and for therapeutic indications in conjunction with the other types of observed behavior.

The continuity and duration of sleep is influenced by mood fixations, content (toxical), support (toxic-metabolic), organic (structural-defect), and personality disorders. The most characteristic finding is early waking in depression. Content disorder tends to produce difficulty in falling asleep. The necessity for a twenty-four hour sleep chart in support and organic disorders is stressed. In personality disorders when insomnia as the principal complaint, we deal with an hypochondriacal or hysterical substitutive reaction which yields best to personality study and general adjustment.

Some hints as to therapy, especially as to drug therapy, are added.

REFERENCES

(1) *Verhandlungen des Deutschen Kongresses für Innere Medizin. 31er Kongress*, 1914, Wiesbaden.

(a) GAUPP, R.: Über Wesen u. Behandlung des Schlaflosigkeit, p. 9–44.

(b) GOLDSCHEIDER: Über Wesen u. Behandlung des Schlaflosigkeit, p. 45–102.

(c) FAUST, E. S.: Über Wesen u. Behandlung des Schlaflosigkeit, p. 103–120.

(d) ROEMHELD, L.: Die Schlaflosigkeit der Dyspeptiker u. ihre Diätetische Beeinflussung, p. 120–127.

(e) CURSCHMANN, H.: Zur Psychotherapie der Schlaflosigkeit, p. 128–139.

(f) KOHNSTAMM, O.: Über gewisse gegen Schlafmittel refraktäre Formen von Schlaflosigkeit, p. 140–144.

(g) MOHR, F.: Die Systematische Psychophysische Behandlung der Schlaflosigkeit, p. 145–150.
(h) FRIEDLÄNDER: Psychasthenia u. Schlaflosigkeit, p. 151–162.
(i) SCHRUMPF, P.: Die Schlaflosigkeit im Hochgebirge, p. 163–168.
(2) LHERMITTE, J., AND A. TOURNAY: Le Sommeil—normal et pathologique. Rapport à la Réunion Neurologique Internationale, Paris, 1924. *Revue Neurol.*, II, (July), 1924.
(3) LHERMITTE, J.: Le Sommeil. Paris, 1931.
(4) SARASON, D.: *Der Schlaf.* München, 1929. Contributions by D. Sarason; O. Pötzl, C. von Economo, E. P. Pick, H. Molitor, A. Straszer.
(5) LAUDENHEIMER, R.: Über Schlafstörungen. *Therapie der Gegenwart*, **32:** 337, 1930.
(6) POLLOCK, H. J.: Disorders of Sleep. *Med. Clin. N. A.*, **13:** 1111, 1930.
(7) GILLESPIE, R. D.: Sleep and the Treatment of its Disorders. London, 1929.
(8) LADAME, CH.: Du Sommeil et de quelques-unes de ses modalités chez les aliénés. *Schweiz. Arch. f. Neur. u. Psychiat.*, **13:** 371, 1923.
(9) LÉVY-VALENSI, J.: Considérations sur les insomnies et leur traitement. *Jour. Méd. Franç.* **15:** 429, 1926.
(10) GUTTMAN, E.: Aktogramme als klinische Schlafkontrolle. *Ztschr. f. d. ges. Neur. u. Psychiat.*, **111:** 309, 1927.
(11) RICHTER, CURT P.: Significance of changes in the electrical resistance of the body during sleep. *Proc. Nat. Acad. Science*, **12:** 214, 1926.
(12) KOHLSCHÜTTER, E.: Messungen der Festigkeit des Schlafes. Leipzig, 1862.
(13) MICHELSON, ED.: Untersuchungen über die Tiefe des Schlafes. Dorpat., 1891.
(14) MACWILLIAM, J. A.: Blood pressures in man under normal and pathological conditions. *Physiol. Review*, **5:** 303, 1925.
(15) KATZENELBOGEN, S.: Calcium content of the brain and its distribution in various organs during diallylbarbituric acid narcosis. *Arch. Neur. and Psychiat.*, **28:** 405, 1932.
(16) WUTH, O.: Klinik und Therapie der Schlafstörungen. *Schweiz. Med. Wchnschr.*, 1931, II, p. 833.
(17) MEYER, ADOLF: Unpublished material used in the introductory course in psychopathology in the Johns Hopkins University.
(18) PÖTZL, O.: in Sarason, "Der Schlaf."
(19) STRASZER, A.: in Sarason, "Der Schlaf."
(20) GILLESPIE, R. D.: in "Sleep," etc., *loc. cit.*, p. 133.
(21) WEISS, P. B.: A comparative study of sedatives employed in psychoneuroses. *Med. J. & Rec.*, **84:** 87, 1931.

UROBILIN EXCRETION OF INFANCY AND CHILDHOOD; RELATION TO BLOOD DESTRUCTION AND FORMATION

HUGH W. JOSEPHS

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland

Submitted for publication February 13, 1934

It has been generally believed that the daily urobilin excretion in the stools bears a relation to the rate of blood destruction. Many attempts have been made to determine some quantitative relation between the haematin derived from blood destruction and the excretion of bile pigments, but the great majority of investigators have not been able to show that such a relationship exists. Thus, Brown, McMaster and Rous (1) determined the bilirubin excretion in dogs whose haemoglobin was falling rapidly but did not obtain the expected amount of bilirubin based on the theory that haematin was changed quantitatively into bilirubin and excreted. Likewise, injections of laked blood have not been followed by a recovery of the expected amount of urobilin in the stools (2, 3), except in the experiments reported by Kuhl (4) who, however, in his work used a method for the determination of urobilin that has since been found to be inaccurate.

It seems reasonable to assume that, other things being equal, the rate of blood destruction will be proportional to the total haemoglobin of the body. If, then, there is a relation between excretion of bile pigments and blood destruction it should be possible to express this relation as a ratio between the bile-pigment excretion and the total haemoglobin. The first purpose of this work is to determine the normal variations of this ratio under different conditions of age, weight, and haemoglobin percentage.[1]

In the work of Brown, McMaster and Rous (1) three dogs were studied during a fall in their haemoglobin. The data for total haemo-

[1] The urobilin-haemoglobin ratio is obtained by dividing the total urobilin excretion, expressed in milligrams, by the grams of circulating haemoglobin.

globin and bilirubin excretion are given in their paper so that it is possible to use their figures to determine the ratio of bilirubin excretion to total haemoglobin. This has been done in table 1. It may be seen that with a range of weight from 23 to 15 kilograms and of haemoglobin from 21.1 to 12.5 grams per 100 cc. the ratios are approximately constant. It is of course not possible to determine bilirubin excretion in the clinic, and we are therefore forced to use urobilin excretion in the stools, although a factor of rather uncertain proportions is introduced, namely, that of the reabsorption of urobilin.

TABLE 1

To demonstrate the constancy of the ratio of bilirubin excretion to total haemoglobin in dogs
(Calculated from the figures of Brown, McMaster and Rous, *J. Exper. Med.*, 37: 746, 1923)

DOG NO.	WEIGHT	HAEMOGLOBIN	TOTAL HAEMOGLOBIN DETERMINED	BILIRUBIN IN BILE	BILIRUBIN ÷ TOTAL HAEMOGLOBIN
	kg.	*gms. per 100 cc.*	*gms.*	*mgs. per day*	
26	23	21.1	441	108	0.24
	22	17.2	330	70	0.21
	20	15.7	279	60	0.22
30	18	14.8	260	60	0.23
	17½	13.7	233	48	0.21
28	15	13.9	168	42	0.25
	15	12.5	161	36	0.22

In table 2 are contained urobilin-total haemoglobin ratios in adults, calculated from figures available in the literature. If the urobilin-haemoglobin ratio is to be useful from a clinical standpoint, it is necessary to be able to rely on calculated rather than on actually determined blood volumes as a basis for the estimation of total haemoglobin. I have, therefore, in table 2 calculated the blood volumes on the assumption of 76 cc. of blood per kilogram of body weight, and have used the calculated values even when the actually determined values were available.

In table 3 are the figures for infants and children. In all these cases the children were normal at the time of the determination. The infants were in the hospital for metabolic studies; the older children

TABLE 2

Calculation of urobilin-haemoglobin ratio from data in the literature

AUTHOR	WEIGHT	HAEMOGLOBIN	BLOOD VOLUME		TOTAL HAEMOGLOBIN FROM CALCULATED BLOOD VOLUME	UROBILIN EXCRETION	UROBILIN-HAEMOGLOBIN RATIO (BLOOD VOL. = 76 CC. × KG.)	
			Calculated	Determined				
	kg.	*gms. per 100 cc.*	*l.*	*l.*	*gms.*	*mgs. per day*		
Paschkis (5)							0.20	
Willy u. Heilmeyer (6)	66	18.5	5.0	5.3	925	152	0.16	
	73	16.6	5.6	5.4	900	147	0.16	
	73	16.0	5.6	5.4	890	161	0.18	
	73	11.8	5.6	5.4	660	110	0.17	
		17.8		5.2	926	115	0.12	
		14.0		5.2	730	87	0.12	
		17.2		8.5	1,460	260	0.18	
		13.0		8.5	1,110	180	0.16	
Belonogowa (7)						120 (average)	0.20	No individual figures
Gitter u. Heilmeyer (2)	65	17.2	4.95		850	111	0.13	
Lichtenstein u. Terwen (8)			4.90			71–193 135 (average	0.18	No individual figures. Average = 70 kg. and 14 gms. Hgb. per 100 cc.
Watson (9)	75	14.2	5.7		810	192	0.24	
	75	15.8	5.7		900	245	0.27	
	70	13.5	5.3		710	217	0.30	
	87	16.8	6.5		1,090	174	0.16	
	51	13.9	3.9		540	54	0.10	
	70	12.9	5.3		710	162	0.23	
	49	14.6	3.7		540	134	0.25	
	80	14.8	6.1		900	149	0.17	
	61	15.0	4.6		690	107	0.16	
	55	13.1	4.2		550	89	0.16	
	75	13.9	5.7		790	122	0.15	
	58	13.5	4.4		600	83	0.14	
	71	14.5	5.4		780	142	0.18	

TABLE 2—*Concluded*

AUTHOR	WEIGHT	HAEMOGLOBIN	BLOOD VOLUME		TOTAL HAEMOGLOBIN FROM CALCULATED BLOOD VOLUME	UROBILIN EXCRETION	UROBILIN-HAEMOGLOBIN RATIO (BLOOD VOL. = 76 CC. × KG.)
			Calculated	Determined			
	kg.	*gms. per 100 cc.*	*l.*	*l.*	*gms.*	*mgs. per day*	
Heilmeyer u. Oetzel (10)	73	16.4	5.5	5.3	905	136	0.15
	66	18.3	5.0	5.4	915	152	0.17
	72	17.0	5.5	6.2	935	167	0.18
	65	17.8	4.9	5.2	870	115	0.13
	62	14.1	4.7	5.5	660	113	0.17
	68	15.5	5.2	6.0	805	110	0.13
	80	17.5	6.1	6.4	1,070	253	0.24
	65	18.5	4.9	5.7	910	137	0.15
	64	17.2	4.9	6.2	845	178	0.21
	67	17.5	5.1	6.8	890	209	0.23
	62	16.4	4.7	5.5	770	195	0.25
	57	17.5	4.3	4.8	755	155	0.21

were either in for study or were convalescent but had been under observation for a sufficient time to insure normal figures. There are unfortunately very few children in the older group.

The method for urobilin determination will be found at the end of the paper.

It is evident that the limits of variation of the ratio are wide. This should scarcely be otherwise considering the number of variable factors that enter into the calculation of the ratio. The normal variation, however, is slight when compared with the variation under pathological conditions, a fact that has been commented on by others. It may be seen that there is a gradual fall in the ratio during childhood. From three months to two years the average lies at about 0.4; from two to twelve years it tends to become lower, reaching a point slightly above 0.2 at twelve years. There are not enough figures to allow a more exact statement. In adults the ratio lies under 0.2. The tendency to a higher ratio in infancy probably corresponds to an increased rate of blood formation as indicated by a reticulocyte percentage higher than that of adults.

The urobilin-haemoglobin ratio has been used recently as an index

TABLE 3

Urobilin excretion and calculation of urobilin-haemoglobin ratios in infants and children

	AGE	WEIGHT	HAEMOGLOBIN	BLOOD VOLUME CALCULATED	TOTAL HAEMOGLOBIN	UROBILIN EXCRETION PER DAY	UROBILIN TO TOTAL HAEMOGLOBIN RATIO	
		kg.	*gms. per 100 cc.*	*cc.*	*gms.*	*mgs.*		
Bu	2 m.	4.8	10.5	365	38.5	30	0.78	
Ch	2 m.	3.4	9.5	260	24.5	13	0.53	
Ke	2 m.	5.3	10.5	405	42.5	22	0.52	
Gr	2½ m.	3.8	10.0	290	29.0	20	0.69	
Ke	3 m.	5.7	11.9	435	51.5	25	0.48	
Hu	3 m.	4.7	9.8	360	35.0	20	0.57	
	3½ m.	5.1	10.9	390	42.5	19	0.45	
Gr	4 m.	4.3	11.2	325	36.5	16	0.44	
Hu	4½ m.	5.0	12.3	380	46.5	25	0.54	
Ho	5 m.	5.4	11.0	410	45.0	18	0.40	
Hou	5 m.	5.0	10.0	380	38.0	20	0.53	
J. S	6½ m.	6.0	9.2	455	42.0	19	0.45	
Ho	6½ m.	6.2	11.3	470	53.0	16	0.30	
Hou	6½ m.	5.7	10.6	435	46.0	20	0.43	Ave. 0.42
Wo	7 m.	6.6	10.4	500	52.0	14	0.27	
W. S	7½ m.	6.7	8.9	510	45.5	21	0.46	
Ho	7½ m.	6.5	11.6	495	57.5	16	0.28	
Hou	7½ m.	6.1	10.0	465	46.5	20	0.43	
Sch	8 m.	6.1	10.7	465	49.5	14	0.28	
H. C	8 m.	7.4	10.0	565	56.5	30	0.53	
Wo	8½ m.	8.2	12.6	625	79.0	22	0.28	
J. S	8½ m.	7.2	7.0	545	38.0	17	0.45	
Sch	9 m.	6.6	11.0	500	55.0	22	0.40	
Sc	10 m.	8	7.4	610	45.0	23	0.51	
Wa	1 yr.	11.6	9.5	870	83.0	30	0.36	
W. T	1 yr.	11.0	8.5	835	71.0	22	0.31	Ave. 0.37
Da	2 yr.	11	11.2	835	92.5	31	0.33	1–2 yr.
Su	2 yr.	10	11.2	760	85.0	42	0.49	
A. S	3 yr.	14	11.0	1,060	117	31	0.26	Ave. 0.32
E. T	5 yr.	18	10.2	1,370	140	40	0.29	3–5 yr.
Fil	4 yr.	15	10.8	1,140	123	50	0.41	
L. D	8 yr.	41	11.3	3,120	352	82	0.23	Ave. 0.22
T. S	9 yr.	43	12.1	3,260	395	88	0.22	6–12 yr.
Pe	12 yr.	45	10.5	3,420	360	80	0.22	

of blood destruction by several investigators (5, 7, 8, 10, 11, 12). Its value for this purpose, however, must be tested empirically, for it does not rest on an actual demonstration of a relationship between the

amount of blood destroyed and the amount of pigment excreted. In a previous paper (13) the attempt was made to test the value of the ratio as an index of blood destruction in cases of anaemia, by balancing it with the reticulocyte percentage considered as an index of blood formation. For this purpose the average normal urobilin-haemoglobin ratio was called the unit of blood destruction, while the average normal reticulocyte percentage was designated as the unit of blood formation. In spite of the fact that the units were admittedly approximations, the attempt to balance the two indices was successful since, when the two processes were about equal as measured in units, the red cells and haemoglobin remained unchanged, whereas, when one or the other process predominated, the red cells and haemoglobin rose or fell as expected.

FACTORS INFLUENCING UROBILIN EXCRETION

There has been a considerable amount of work done in a study of factors influencing urobilin excretion, especially types of food and variations in intestinal motility. The influence of food will not be considered here. It has been stated that constipation decreases and diarrhea increases the output of urobilin (12, 14, 15). This is explained on the hypothesis that in constipation urobilin absorption is increased while in diarrhea it is decreased. My experience with infants and young children has been different, for in them diarrhea decreased the urobilin excretion markedly. On the other hand, retention of faeces in small children is only in rare instances of sufficiently long duration to cause any considerable reduction of urobilin in the stools. Only in older children has there been any significant reduction of urobilin excretion due to retention of faeces.

Of greater interest is the relation of urobilin excretion to increased haematopoietic activity, or to conditions in which there is a need for new haemoglobin formation. It has been found that, after withdrawal of blood and at other times when it was probable that the organism was forming haemoglobin, a reduction in the urobilin-haemoglobin ratio took place (4, 6, 16, 17). This was interpreted as a "sparing action," by which the products of blood destruction were retained for the purpose of new haemoglobin formation.

TABLE 4

Course of urobilin excretion and urobilin-haemoglobin ratio following pneumonia in infants

NAME	AGE	DAYS AFTER FALL IN TEMPERATURE	WEIGHT	HAEMOGLOBIN	HAEMOGLOBIN TOTAL	UROBILIN EXCRETION	UROBILIN TO TOTAL HAEMOGLOBIN RATIO	RETICULOCYTES
			kg.	*gms. per 100 cc.*	*gms.*	*mg.*		
St.	7 m.	0	8.5	10.0	64	85	1.3	1
		2	8.5	10.5	66	38	0.56	2
		4	8.5	10.2	65	10	0.15	3
		8	8.5	10.2	65	12	0.18	1.5
Ho.	15 m.	0	8.7	5.0	34	125	3.4	1
		4	8.9	6.2	42	63	1.5	5
		10	9.0	6.6	45	45	0.55	5
		15	9.5	7.1	51	46	0.90	3
Wa.	6 m.	−3	7.0	8.6	46	35	0.76	0.5
		5	6.9	10.3	54	10	0.18	1.5
		10	6.9	10.4	55	20	0.36	2.0
Ma.	19 m.	0	9.3	6.3	44	90	2.02	occas.
		2	9.3	6.9	48	35	0.72	2.5
		5	9.3	7.7	54	12	0.22	6
		10	9.3	8.4	59	40	0.67	3
Yo.	10 m.	0	7.8	6.5	38.5	32	0.83	0
		4	7.8	6.0	35.5	38	1.07	3
		7	7.8	6.5	38.5	33	0.86	2
Co.	8 m.	0	6.9	7.5	38	25	0.7	0
		3	6.5	8.4	40	8	0.20	1.5
		9	6.5	8.3	40	90	2.2	5
Sc.	9½ m.	−1	7.0	8.0	42	25	0.59	occas.
		1	7.0	8.0	42	10	0.24	1
		3	7.0	7.8	41	16	0.39	4
		10	7.0	8.0	42	23	0.55	1
Co.	1 yr.	−3	9.0	5.6	38	52	1.4	0
		0	8.9	5.6	37	10	0.27	occas.
		10	8.8	5.6	37	32	0.86	1

INFECTIONS

During infections in infancy there frequently occurs a considerable fall in haemoglobin accompanied usually by an increased urobilin

excretion. The period immediately following an infection seemed to be one in which such a "sparing" action might possibly be demonstrated. Table 4 contains the results of determinations of urobilin immediately following pneumonia. As may be seen, at about the time the fever subsided there was a rapid fall in urobilin excretion which sometimes reached very low figures. In one or two cases the amount of urobilin excreted was so small as to be almost negligible for a short time. The periods in these cases were of two days each.

TABLE 5

Case of anaemia of haemolytic origin treated with liver and later with iron

DATE	WEIGHT	HAEMOGLOBIN	HAEMOGLOBIN TOTAL	UROBILIN EXCRETION	UROBILIN TO TOTAL HAEMOGLOBIN RATIO	RETICULOCYTES	
	kg.	*gms. per 100 cc.*	*gms.*	*mgs.*		*per cent*	
5/31–6/ 2/29	6.8	6.1	31	240	7.7	5	Liver begun 5/31
6/ 2–6/ 5/29	6.8	6.1	31	320	10.3	10	
6/ 7–6/10/29	6.9	7.0	37	400	10.8	14	
6/10–6/13/29	6.9	7.2	38	230	6.1		
6/13–6/15/29	7.0	7.4	39	280	7.2	7	
6/15–6/17/29	7.1	7.7	42	235	5.6	2.5	
6/17–6/19/29	7.1	8.0	43	135	3.1		Liver discontinued. Iron begun 6/16
6/19–6/21/29	7.2	8.0	44	185	4.2	2	
6/21–6/23/29	7.2	7.8	43	300	7.0	7.5	
6/24–6/26/29	7.3	7.7	43	255	5.9		
6/26–6/28/29	7.3	7.5	42	210	5.0	7	
6/29–7/ 1/29	7.4	8.5	48	160	3.3	5.5	
7/ 1–7/ 3/29	7.5	8.9	51	110	2.5		
7/ 3–7/ 6/29	7.6	8.9	51	50	0.98	8	

(In infancy it is not necessary to use longer periods than this.) It may be seen that the ratios in many cases reached points considerably below the normal for the age. The period of decreased excretion was, however, of short duration and was often followed by a rise which was accompanied by a rise in reticulocytes. Whether the low figures following infection mean a retention of pigments derived from blood destruction or a decrease in the rate of blood destruction itself to a subnormal level, it is impossible to say. Whatever the explanation, there can be no doubt of the presence of a conservative mechanism following pneumonia, lasting for a comparatively short period.

TABLE 6

Course of urobilin excretion and urobilin-haemoglobin ratio after administration of iron in cases of anaemia in infancy

		WEIGHT	HAEMOGLOBIN	HAEMOGLOBIN TOTAL	UROBILIN EXCRETION	UROBILIN TO TOTAL HAEMOGLOBIN RATIO	RETICULOCYTES
		kg.	*gms. per 100 cc.*	*gms.*	*mgs. per day*		*per cent*
1	Before iron	6.7	5.5	28	82	2.9	2–4
	After iron (1st week)	6.7	6.2	32	78	2.4	10
	(2nd week)	6.7	7.4	38	72	1.9	4
	(3rd week)	6.7	8.8	45	40	0.89	3
2	Before iron	6.7	8.2	42	70	1.65	2
	After iron (1st week)	7.1	8.2	44	75	1.70	6
	(2nd week)	7.3	8.8+	49	35	0.71	1
3	Before iron	4.3	4.2	13.5	15	1.10	3
	After iron (1st week)	4.3	4.6	15	15	1.00	10
	(2nd week)	4.8	6.7	24	50	2.1	10
	(3rd week)	5.2	8.0	32	65	2.1	5
4	Before iron	8.6	5.3	35	30	0.86	1–2
	After iron (1st week)	8.6	5.6	37	105	2.8	6
	(2nd week)	8.6	6.5	43	70	1.6	4
	(3rd week)	8.7	6.7	44	55	1.25	5
5	Before iron	9.6	7.0	51	46	0.90	3
	After iron (1st week)	9.6	7.4	54	52	0.96	12
	(2nd week)	9.8	9.0	67	92	1.35	2–3
	(3rd week)	9.9	9.6	72	50	0.69	1
6	Before iron	8.1	3.9	24	37	1.55	4
	After iron (1st week)	8.4	4.6	29	50	1.75	10
	(2nd week)	8.4	6.3	40	82	2.05	12
	(3rd week)	8.7	9.0	59	60	1.00	4
7	Before iron	8.2	3.8	24	37	1.55	4
	After iron (1st week)	8.0	4.3	26	105	4.0	9
	(2nd week)	8.1	8.0	49	65	1.35	12–14
	(3rd week)	8.2	9.4	59	33	0.56	10–7
8	Before iron	7.5	8.0	46	35	0.76	1–2
	After iron (1st week)	7.5	8.6	49	21	0.43	1–2
	(2nd week)	7.5	8.8	50	21	0.42	1–2

TABLE 6—*Concluded*

		WEIGHT	HAEMOGLOBIN	HAEMOGLOBIN TOTAL	UROBILIN EXCRETION	UROBILIN TO TOTAL HAEMOGLOBIN RATIO	RETICULOCYTES
		kg.	*gms. per 100 cc.*	*gms.*	*mgs. per day*		*per cent*
9	Before iron	6.8	7.5	39	32	0.82	4
	After iron (1st week)	8.8	8.4	44	40	0.91	2
	(2nd week)	8.8	8.6	45	40	0.89	2
10	Before iron	9.3	6.7	47	22	0.47	2
	After iron (1st week)	9.5	7.7	56	55	0.98	10
	(2nd week)	9.5	9.5	69	35	0.51	3
11	Before iron	9.0	6.3	43	80	1.85	2
	After iron (1st week)	9.5	6.6	48	160	3.3	9
	(2nd week)	10.0	7.7	59	85	1.45	5
	(3rd week)	10.5	8.4	67	50	0.75	1
12	Before iron	10.2	3.0	23	50	2.2	5
	After iron (1st week)	10.2	3.7	29	55	1.90	7
	(2nd week)	10.2	5.6	43	120	2.8	10
	(3rd week)	10.2	7.1	55	45	0.82	4
	(4th week)	10.2	8.4	65	40	0.62	2

STIMULATION OF HAEMATOPOIESIS WITH LIVER OR IRON

Singer (3) and Lichtenstein and Landsberg (18) found that in normal individuals large doses of liver caused a rise in urobilin excretion. The fall in urobilin excretion resulting from liver therapy in pernicious anaemia has been ascribed to the fact that liver causes the formation of red cells that are better able to resist haemolysis (3, 7, 12). In a case of anaemia of haemolytic origin which ran a rather prolonged course during convalescence from pneumonia, it was found that liver caused a rise both in urobilin excretion and in reticulocytes (table 5).

A rise in urobilin excretion accompanying a rise in reticulocytes when the haematopoietic tissue is stimulated appears to be a phenomenon of fairly regular occurrence. In experimental work the injection of haemolyzed blood was sometimes followed by an excretion of urobilin greater than could be accounted for by the blood pigments injected. In these cases there was also evidence of increased blood

formation and a slight but definite rise in haemoglobin (2, 4). In the anaemia of infancy, iron has a marked stimulating effect on blood formation. In table 6 are contained observations on the urobilin excretion and reticulocyte rise in cases of anaemia treated with iron. In eight of the twelve cases a definite rise in urobilin-haemoglobin ratio took place, and in all of these there was a rise in reticulocytes. In two cases (8 and 9) there was no rise in either urobilin excretion or in the reticulocytes. In the remaining two the relationship did not hold (1 and 2). In one of these the urobilin excretion at the time that iron was given was very high and in the other moderately high, and it is possible that the high urobilin excretion masked the effect of iron. It is difficult to analyze this result of the administration of iron. It is not probable that iron causes primarily an increase in blood destruction and that the rise in reticulocytes is secondary to the increased haemolysis. Two other possibilities are more likely: (1) That iron causes an output of young cells which are more subject to haemolysis than is normally the case; (2) That iron may cause stimulation of the total blood metabolism, *i.e.*, both the anabolic and the katabolic processes. Until we know more about these processes this interesting effect of iron must remain unexplained.

SUMMARY

Although variations in urobilin excretion have for a long time been considered as measuring changes in the rate of blood destruction, it is only recently that there has been any quantitative expression of the relationship of urobilin excretion to blood destruction. This has taken the form of a ratio between the daily urobilin excretion and the total haemoglobin of the body. In this paper the relative constancy of this ratio at different ages has been demonstrated. Certain factors influencing the ratio have been discussed.

1. The ratio tends to be slightly higher in the first two years of life than later in the childhood. 2. An increase during pneumonia is followed by subnormal values which last for a short time early in convalescence. 3. Stimulation of the haematopoietic tissue by iron is accompanied in the majority of cases by an increase in urobilin-haemoglobin ratios. In one case liver also caused a rise in urobilin excretion accompanying stimulation of blood-forming tissue. It was

suggested that these stimulants might affect the total metabolism of the blood, not merely the anabolic constituent.

METHOD FOR UROBILIN DETERMINATIONS (ELMAN AND McMASTER (18), SLIGHTLY MODIFIED)

Stools. The specimens were sent to the laboratory as they were passed, in the bedpan, if older children were being investigated; in the diapers, if infants. They were then placed in the refrigerator until the determination was to be made. It was found that specimens could be kept for at least four or five days without deterioration, but in practice three days was about as long as any stool was kept before being examined. The specimens from two or three days were added together and a single determination was made for the entire period. In the case of very young babies, however, in which there were small amounts of stools on each of several diapers, the danger of drying out was greater and estimations were made every day. The stools were transferred to a porcelain dish, water was added and the material ground up under the water to make an emulsion. This was put through a fine strainer into a beaker, then more water was added to the dish and the stool remaining in the dish and strainer was ground and strained. This process was repeated until finally all the stool was in the beaker in the form of a fine emulsion. The total amount was measured, and a 10 cc. portion transferred to a 50 cc. stoppered cylinder, care being taken that the emulsion was well stirred before being transferred. Acid alcohol (30 cc.) was added, the cylinder shaken once or twice and allowed to stand overnight in the dark. The next day 5 cc. of the acid alcohol emulsion, after shaking to mix thoroughly, were transferred to a 10 cc. cylinder, a drop of tincture of iodine and then a small amount of powdered zinc acetate was added and the mixture shaken for a minute or so to dissolve the zinc acetate. A saturated solution of zinc acetate in alcohol was added to the 10 cc. mark and the mixture filtered. The filtrate should be clear and fluorescent. This fluorescent filtrate was diluted until the fluorescence was the same as that of a 1–30,000,000 solution of acriflavine, as follows: Two test-tubes of equal bore were taken; into one was put 15 cc. of the 1–30,000,000 solution of acriflavine freshly prepared from the stock solution, into the other 15 cc. standard diluting fluid (see below). The fluorescent filtrate was added to the standard diluting fluid until its fluorescence matched that of the standard. The matching of the fluorescence was done by means of a box one end of which was open, while at the other were two holes bored in the top into which the two test-tubes would just fit. The tubes were placed so that a strong beam of artificial light would shine vertically through the length of the tubes when the observer looked at the tubes in a horizontal direction, as far as possible preventing light from entering the box at the open end.

Solutions: Acid alcohol: 1600 cc. 95 per cent alcohol; 25 cc. concentrated HCl. Water to 2500 cc.

Saturated zinc acetate in alcohol, made up fresh every few days.

Standard diluting fluid. 2000 cc. 60 per cent alcohol; 50 grams zinc acetate. 10 cc. concentrated HCl filtered until clear.

Standard acriflavine. A 1–1000 solution of acriflavine was made up and kept in the refrigerator. From this a 1–100,000 solution was made up every few months. A 1–10,000,000 solution was prepared every three days and from this the 1–30,000,000 solution was freshly made each time a series of determinations was carried out. Both the 1–100,000 and 1–10,000,000 solutions were kept in the refrigerator.

Procedure for urine. The urine was measured, if alkaline, it was made slightly acid and 5 cc. added to a 10 cc. cylinder. Two drops of tincture of iodine were added, the procedure from this point being the same as for the stools, except that a filter paper must be used that is capable of holding back finer particles than in the case of stools.

The results thus far are in the form of dilution figures. To obtain the figures for urobilin in milligrams it is necessary to know what dilution of urobilin will give the same amount of fluorescence. For this purpose urobilin was prepared as outlined by Elman and McMaster and this was used to standardize the method. It was found that 1 milligram of urobilin so prepared when diluted to 2 litres with the standard diluting fluid would give the same fluorescence as a 1–30,000,000 solution of acriflavine.

The calculation of milligrams of urobilin then is a simple matter. For stool it is

$$\frac{\text{cc. stool emulsion} \times 4 \times 2 \times \dfrac{15 + \text{cc. fluorescent filtrate}}{\text{cc. fluorescent filtrate}}}{2000^{2}} = \text{milligrams of urobilin}$$

For example, the stool emulsion measured 200 cc. This was diluted three times with acid alcohol (total dilution now 800). The acid alcohol emulsion was doubled by the addition of saturated zinc acetate (dilution now 1600). 0.3 cc. of the fluorescent filtrate was used to add to the 15 cc. of standard diluting fluid to obtain the proper amount of fluorescence (dilution $= 1600 \times \frac{15.3}{.3} = 81{,}600$). This figure divided by 2000 = 41 milligrams in the stool specimen.

For urine the calculation is similar except that the factor 4 is not present.

If much urobilin is present in the stools it may be necessary to dilute the fluorescent filtrate before adding to the standard diluting fluid to compare the fluorescence with the standard. This dilution should be done with the standard diluting fluid.

---

[2] This figure for conversion of the dilution value to milligrams is different from that of Elman and McMaster. They say nothing of the use of freshly made up saturated zinc acetate. Using a zinc acetate solution that had stood for more than a month I obtained the factor that they gave in their article.

Precautions. The procedure should be carried out with as little variation as possible. It is probably best to standardize the method with a known urobilin solution and then never vary the procedure.

The zinc acetate solution should be freshly prepared every few days. It deteriorates on standing for a longer period.

The test-tubes used in the comparison of fluorescence should be free of any grease or scratch marks. These reflect the light and interfere with the fluoresence.

Remarks on Urobilin Excretion

The term "urobilin," as used in this paper, covers a number of substances of unknown composition giving fluorescence in alcoholic zinc acetate.

Duration of periods. It is usually stated that periods should be of at least four days. This is true for older children. In infants two-day periods are quite satisfactory especially if repeated. No conclusions can be drawn from a single day period at any age. It is, however, unsafe to draw conclusions from a single period, even of four days unless it is known that the child is quite normal. Several consecutive periods to obtain the direction of the change in excretion or to determine that there is no significant change are desirable.

Significant increases are large—100 to 200 per cent or more—hence slight increases up to say 50 per cent should not be considered too seriously. In haemolytic anaemias, the amount of urobilin excretion per day may be as much as twenty to thirty times the normal.

Urine. In computing total urobilin, the amount excreted in the urine can be disregarded, being only 5 per cent of the total, except in cases in which there is extensive liver damage, when it may amount to 10–20 per cent or more.

BIBLIOGRAPHY

1. Brown, G. O., McMaster, P. D. and Rous, P.: *J. Exper. Med.*, **37:** 733, 1923.
2. Gitter, A., u. Heilmeyer, L.: *Ztschr. f. d. ges. exper. Med.*, **77:** 594, 1931.
3. Singer, K.: *Ztschr. f. d. ges. exper. Med.*, **71:** 137, 1930.
4. Kuhl, G.: *Arch. f. Exper. Path. u. Pharmakol.*, **103:** 247, 1924.
5. Paschkis, K.: *Ztschr. f. klin. Med.*, **116:** 680, 1931.
6. Willy, O., u. Heilmeyer, L.: *Ztschr. f. d. ges. exper. Med.*, **77:** 144, 1931.
7. Belonogowa, N. S.: *Deutsches Arch. f. klin. Med.*, **162:** 297, 1928.
8. Lichtenstein, A., u. Terwen, A., J. L.: *Deutsches Arch. f. klin. Med.*, **149:** 102, 1925.
9. Watson, C. J.: *Arch. Int. Med.*, **47:** 698, 1931.
10. Heilmeyer, L., u. Oetzel, W.: *Deutsches Arch. f. klin. Med.*, **171:** 365, 1931.
11. Sonnenfeld, A.: *Ztschr. f. klin. Med.*, **100:** 508, 1924.
12. Paschkis, K., u. Diamant, M.: *Deutsches Arch. f. klin. Med.*, **169:** 180, 1930.
13. Josephs, H.: *Bull. Johns Hopkins Hosp.*, **51:** 185, 1932.

14. McMaster, P. D., and Elman, R.: *J. Exper. Med.*, **41**: 513, 719, 1925. Elman, R., and McMaster, P. D.: *ibid.*, **42**: 99, 619, 1925.
15. Belonogowa, N. S.: *Deutsches Arch. f. klin. Med.*, **170**: 436, 1931.
16. Paschkis, K.: *Ergebn. d. inn. Med. u. Kinderh.*, **45**: 682, 1933.
17. Otto, W., u. Heilmeyer, L.: *Ztschr. f. d. ges. exper. Med.*, **77**: 144, 1931.
18. Elman, R., and McMaster, P. D.: *J. Exper. Med.*, **41**: 503 (1925).

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this journal.)

Benign Tumors in the Third Ventricle of the Brain: Diagnosis and Treatment. By WALTER E. DANDY. 171 pp. Illust. (*Charles C. Thomas, Springfield, Ill., and Baltimore, Md., 1933.*)

This monograph deals with an entirely new phase of neurological surgery—the diagnosis, localization and operative treatment of benign tumors within the third ventricle. To the knowledge of this reviewer there is not another case of this type in the literature that was correctly diagnosed or disclosed by operation before death. Dr. Dandy presents 21 of these cases, diagnosed and accurately localized before he made his surgical attack, which was attended by a mortality rate of less than 15 per cent in the last fourteen cases, and of 33 per cent in all the cases. Ventriculography is the one outstanding procedure that has helped rescue these cases from a necropsy diagnosis.

When one has finished reading this monograph one realizes that another milepost in neurological surgery has been set up and the way paved clearly and carefully for others to follow. In keeping with this monograph are the splendid illustrations and the fine printing.

F. W. G.

The Pregnant Woman. By PORTER BROWN. 174 pp. (*Eugenics Publishing Co., Inc., New York, 1933.*)

An exeellent exposé of female physiology and psychology for uninformed femininity, including female adolescents. Of no value for the obstetrician.

M. J.

Sex Habits. A Vital Factor in Well-Being. By A. BUSCHKE and F. JACOBSOHN. 204 pp. (*Emerson Books, Inc., N. Y., 1933.*)

A philosophical and ethical essay on a weighty problem, with attempts at advice. A bit radical in some of its precepts from the practical standpoint, and a bit partial to the masculine outlook. Of interest to the professional man, but fraught with danger for the adolescent and the lay adult.

M. J.